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Heavy metals and plant uptake of metals in agricultural soils of Kosovo

zur Erlangung des Doktorgrades (Dr. agr.)
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Kumulative Dissertation

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Abstract

Heavy metals occur naturally in soils; some are essential micronutrients for plants growth and are thus important for human health and food production. Depending on content and availability in soil, however, they become potentially toxic. Regardless of the dependence of heavy metals bioavailability on soil properties, in the legislation of many countries (including Kosovo), the maximum permitted value (MPV) of heavy metals is determined based on pseudo total metal concentration. Therefore, it is very important to consider the influence of soil properties in the bioavailability of metals in MPV determination.

This thesis is separated into two parts. The aim of the first part is to evaluate the metal content in agricultural soils of Kosovo, regarding metal bioavailability and soil properties. This main aim is achieved through several sub-targets: a) Determination of pseudo total heavy metals in agricultural soils of Kosovo, b) Determination of the potential bioavailability form and mobile form of metals, and c) Use of regression models to investigate the influence of different soil properties on bioavailable forms of metals.

The objective of the second part is to assess the metal contents in agricultural soils and different plants in two more contaminated regions of Kosovo. This objective is realized by several sub-objectives: a) Determination of pseudo total heavy metals and their bioavailability form in agricultural soils, b) Determination of metal contents in different plants, c) Assessment of metal transfer factor from soil to plants, and, d) Developing regression models to predict plant metal uptake using soil properties.

Based on these, in the first part, 127 topsoil samples were collected from all agricultural sites of Kosovo, whereas in the second part, there were collected 60 soil and plant samples (wheat, corn, potatoes and grass) from two regions of Kosovo (Drenas and Mitrovica). Heavy metals were extracted from soil with aqua-regia (pseudototal concentration), NH_4Oac-EDTA (potential bioavailable) and NH_4NO_3

(mobile fraction), while plant samples were digested with HNO_3/H_2O_2 (microwave assisted extraction).

Concentrations of Ni showed that 62% of the soil samples were above threshold values, whereas increased values for Pb, Cd, Zn and Cr were in 9%, 6%, 5%, and 2% of the sample set, respectively. Only Cu was below threshold values in all analyzed samples. Nevertheless, the percentage of potential bioavailable (PBF) and mobile forms (MF) of Ni showed low value (mean 6.9, 0.53 respectively), whereas for Cd and Pb it showed higher values. In order to assess the bioavailability of heavy metals, relevant soil parameters were determined. Regarding mobile fractions of HM, only Ni was significantly influenced by its total concentrations. For most of HM in mobile fractions, soil pH significantly impacted the extracted metal amounts.

The comparison between the two considered more contaminated regions in Kosovo has shown higher values in Mitrovica (mean: Cd - 2.92, Pb - 570.15, and Zn - 522.86 mg kg^{-1}) for pseudo total contents of Cd, Pb, and Zn. The same has been found for the potential bioavailability and mobile form of these metals (mean: Cd – 1.59, Pb - 217.05, Zn - 522.86 mg kg^{-1}, respectively Cd - 0.17, Pb - 0.64, and Zn - 15.45 mg kg^{-1}). Cd and Pb were elevated in potato tubers (mean Cd - 0.48 and Pb – 0.85 mg kg^{-1}) and can be dangerous for human health. The multiple regression analysis showed a good model for prediction of Cd, Pb and Zn contents in plants with significance 99.9%, whereas this model was not significant for Cu, Cr and Ni. Soil pH played a significant role in Cd and Zn contents in wheat and potato plants. Clay content also showed significance in Cd concentrations in wheat and potato plants, while carbon content was significant for Cd in grass plants, as well as for Zn in wheat and grass plants.

Zusammenfassung

Schwermetalle kommen natürlicherweise in Böden vor; einige sind wesentliche Mikronährstoffe für das Pflanzenwachstum und sind so für die menschliche Gesundheit und Nahrungsmittelproduktion wichtig. Abhängig von ihrer Konzentration und Verfügbarkeit im Boden sind einige von ihnen potenziell toxisch. In der Gesetzgebung vieler Länder (einschließlich des Kosovo), wird der maximal erlaubte Wert (MPV) von Schwermetallen im Boden auf die Pseudogesamtmetallkonzentration bezogen. Die tatsächliche Verfügbarkeit der Metalle für eine Aufnahme in die Pflanze wird dabei nicht betrachtet. Deshalb ist es sehr wichtig, den Einfluss von Bodeneigenschaften auf die Bioverfügbarkeit von Metallen bei der MPV-Bestimmung zu betrachten.

Diese Doktorarbeit gliedert sich in zwei Teile. Das Ziel des ersten Teils ist, den Metallgehalt in landwirtschaftlichen Böden des Kosovo, betreffend der Metallbioverfügbarkeit und Bodeneigenschaften auszuwerten. Dieses Hauptziel wird durch folgende Unterziele erreicht: a) Bestimmung von Pseudogesamtmetallgehalten in landwirtschaftlichen Böden des Kosovo, b) Bestimmung der potenziellen bioverfügbaren- und mobilen Metallgehalte c) Verwendung von Regressionsmodellen, um den Einfluss von verschiedenen Bodeneigenschaften auf den Anteil bioverfügbarer Metalle zu untersuchen.

Das Ziel des zweiten Teils ist es, Metallgehalte in landwirtschaftlichen Böden und in verschiedenen Pflanzen aus zwei belasteten Regionen des Kosovo festzusetzen. Dieses Ziel wird durch folgende Unterziele verwirklicht: a) Bestimmung von Pseudogesamt- und bioverfügbaren Metallgehalten in landwirtschaftlichen Böden, b) Bestimmung der Metallgehalte in verschiedenen Pflanzen c) Abschätzung der Metalltransferfaktoren vom Boden in die Pflanze, und, d) Entwicklung von Regressionsmodellen, um den Einfluss von verschiedenen Bodeneigenschaften auf den Pflanzenmetallgehalt zu untersuchen

Für den ersten Teil wurden 127 Oberbodenproben aller relevanter landwirtschaftlicher Standorte im Kosovo gesammelt. Für den zweiten Teil wurden

60 Boden- und 60 Pflanzenproben (Weizen, Getreide, Kartoffeln und Gras) in zwei Gebieten des Kosovo (Drenas und Mitrovica) genommen. Die verschiedenen Metallfraktionen im Boden wurden mit Mikrowellen unterstützer Königswasser (Pseudogesamtmetalgehalt), NH_4Oac-EDTA Extraktion (potenziell bioverfügbarer Metallgehalt) und NH_4NO_3 Extraktion (mobiler Metallgehalt) bestimmt. Die Pflanzenproben wurden einem Mikrowellenextraktionsverfahren (HNO_3, H_2O_2) unterzogen.

Konzentrationen von Ni haben gezeigt, dass 62% der Bodenproben Grenzwerte überschritten, wohingegen erhöhte Werte für Pb, Cd, Zn und Cr in 9%, 6%, 5 % und 2% des Probensets vorkamen. Lediglich für Cu wurden keine Grenzwertüberscheitungen in allen analysierten Proben festgestellt. Die Anteile der potenziell bioverfügbaren (PBF) und mobilen Formen (MF) von Ni zeigten niedrige Werte (6,9 und 0,53%), während für Cd und Pb höhere Werte ermittelt wurden. Um die Bioverfügbarkeit von Schwermetallen festzusetzen, wurden relevante Bodenparameter bestimmt. Bezüglich des mobilen Metallgehalts, wurde lediglich Ni erheblich durch seine Gesamtkonzentrationen beeinflusst. Für die meisten mobilen Metalle wirkte sich der pH-Wert des Bodens erheblich auf die extrahierten Metallmengen aus.

Der Vergleich zwischen den zwei betrachteten belasteten Regionen im Kosovo hat höhere Metallkonzentrationenen in Mitrovica gezeigt (Durchschnitt: Cd - 2,92, Pb - 570,15 und Zn – 522,86 mg kg^{-1}) für den Pseudogesamtgehalt Cd, Pb und Zn. Dasselbe ist für die potenziell bioverfügbaren und mobilen Metallgehalte gefunden worden (Cd – 1,59, Pb – 217,05, Zn – 522,86 mg kg^{-1}, bzw Cd – 0,17, Pb – 0,64, und Zn – 15,45 mg kg^{-1}). Cd und Pb Konzentrationen waren in Kartoffelknollen erhöht (Cd – 0,48 und Pb – 0,85 mg kg^{-1}), und können für die menschliche Gesundheit gefährlich sein. Die Mehrfach-Regressionsanalyse zeigte ein gutes Modell zur Vorhersage des Cd, Pb und Zn Gehalts in den Pflanzen mit hoher Signifikanz während dieses Modell für Cu, Cr und Ni nicht signifikant war. Der pH-Wert des Bodens hat eine bedeutende Rolle für Metallgehalte (Cd und Zn) in Weizen und in der Kartoffel gespielt. Der Tongehalt hat signifikanten Einfluss auf die Cd

Konzentrationen in Weizen und Kartoffeln, während der Kohlenstoffgehalt für Cd in Gräsern, sowie für Zn in Weizen und Gras Pflanzen von Bedeutung war.

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Abbreviations

AAS	Atomic Adsorption Spectrophotometer
AEC	Anion Exchange Capacity
AR	Aqua Regia
BMU	Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (Bundesministerium für Umwelt, Naturschutz, Bau und Reaktorsicherheit)
C	Carbon
CEC	Cation Exchange Capacity
CRM	Certified Reference Material
CSS	Composite soil sampling,
GP	Generative part
HM	Heavy metals
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometer
JLU	Justus Liebig University
M	Metal
M(AN)	Metal extracted by ammonium nitrat
M(AR)	Metal extracted by-*aqua regia*
M(EDTA)	Metal extracted by -EDTA
MAE	Microwave Assisted Extraction
MF	Potential mobility factor
MP	Metals in plant
Ms	Metals in soil

OM	Organic matter
PBF	Potential bioavailability factor
REI	Relative explanation index
RM	Reference Material
SSS	Single soil sampling
TF	Transfer Factor
TFSG	Transfer factor soil – generative part
TFSV	Transfer factor soil – vegetative part
VP	Vegetative part
WRB	World Reference Base for Soil Resources

List of Publications related to doctoral thesis

- Zogaj, M., Paçarizi, M., Düring, R-A. (2013): The correlation between heavy metals concentration in soil and plants in the municipality of Drenas. Papers II, from annual science conference "Science Week 2013" , ISBN 978-9951-16-058-2, Vol. 2, 97-103, Ministry of Education, Science & Technology of Kosovo. (In Albanian).
- Zogaj, M., Paçarizi, M., Düring, R-A. (2014): Spatial distribution of heavy metals and assessment of their bioavailability in agricultural soils of Kosovo, *Carpathian Journal of Earth and Environmental Sciences*, Vol. 9, No. 1, p. 221 – 230
- Zogaj, M. and Düring, R-A. (2016.): Plant uptake of metals, transfer factors and prediction model for two contaminated regions of Kosovo, *J. Plant Nutr. Soil Sci.* 2016, 000, 1–11 DOI: 10.1002/jpln.201600022

Conference contributions related to doctoral thesis

- Zogaj, M., Kamberi, M., Paçarizi, M., Düring, R-A. (2013): Bioavailability of Heavy Metals in different land use in Drenica region, Kosovo. EGU Conference, Vienna, Austria (Poster).
- Zogaj, M., Paçarizi, M., Düring, R-A. (2013): The correlation between heavy metals concentration in soil and plants in the municipality of Drenas. Science week of Kosovo, Book of abstracts, ISBN 978-9951-16-053-7 13-18 May, Ministry of Education, Science & Technology of Kosovo. (In Albanian), (Oral Presentation).
- Zogaj, M. and Düring, R-A. (2014): Hevay metal contents in soil and potato tubers in Mitrovica region. First international scientific symposium of agriculture and veterinary medicine, Prishtinë. (In Albanian), (Oral Presentation).

- Zogaj, M. and Düring, R-A. (2015): Vertcal distribution of heavy metals in agricultural soil profiles in two regions of Kosovo, International conference on soil, Tirana. (Oral Presentation).
- Zogaj, M. and Düring, R-A. (2016): Potential ecological risk assessment of heavy metals for agricultural soil of Drenas Municipality, International Conference of the DAAD Biodiversity Network Project 'Agriculture and biodiversity on the Balkan Peninsula'. Prishtine (Poster).

Extended Summary

1.1 Introduction

1.1.1 General introduction

Soil is a non-renewable natural source and generally defined as the top layer of the earth's crust, formed by mineral particles, organic matter, water, air and living organisms. It has several ecological functions: a) providing food and biomass (storing, filtering and transforming several substances - water, nutrients and carbon); b) maintaining biodiversity; c) physical and cultural environment for humans (providing raw materials, archiving geological and archeological heritage) (Commission of the European Communities, 2006). Therefore, any human activity affecting soil needs to be conducted with caution making sure that soil preserves its ecological function (Ivezić, 2011).

The term "heavy metals" and/or "Trace metals/elements" have been widely used in literature recently. However, it is not so simple to define the term "heavy metals". What is "heavy"? There is no standard definition assigning metals as heavy metals. According to Appenroth (2010), this definition is meant to suggest that the density of a heavy metal is high, but in the context of plants and other living organisms, it is quite meaningless and the density of the metal does not play any role. Some lighter metals and metalloids (e.g. arsenic) are toxic and thus are termed heavy metals, while some heavy metals, such as gold typically, are not toxic. Duffus (2002), found 13 different studies being cited that used lower limits on the density of a "heavy" metal ranging from 3.5 to 7 g cm^{-3}. However, while there is no reclassification of metals, we have continued to use terms “metals” and “heavy metals” in this doctoral thesis.

Kosovo, located in the center of Balkan Peninsula (N 43° 16’ – 41° 53’ and E 21° 16 – 19° 59’), represents a country of great interest for studies on behavior of metals in contaminated soils. In fact, it is mining and industrial activities, which are located in different parts of eastern Kosovo, that mostly cause contamination of the environment and agricultural soils. The following heavy industry sites are found in the area: the ore-metallurgic combine “Trepça” in Mitrovica, the Kosovo Energetic Corporation in Obiliq, “Ferronikeli” in Drenas, the Battery Factory Ni-Cd “IBG-Gjilan”, „Cementorja“ Hani i Elezit. Some authors report high levels of heavy metals in areas close to these contamination sources (Zogaj et al., 2014; Šajn et al., 2013; Nanoni et al., 2011; Borgna et al., 2009), which have passed the permitted value for soil many times regarding EU standards.

1.1.2 Sources of metals

Metals can be introduced to the agricultural soil from both natural and anthropogenic sources. Heavy metals occur naturally in soils due to pedogenetic and biochemical processes of weathering parent materials. Even though the concentrations of these metals are regarded as trace (<1000 $mg \cdot kg^{-1}$) and rarely toxic (Boroń and Boroń, 2014), most of the concerning contaminated centers are found around anthropogenic sources (Bergsten, 2006). Depending on the geology of the parent material from which the soil was formed, the content of metals in uncontaminated soil is widely distributed. According to Lindsay (1979), the common range of metal contents in natural soils are: Cd 0.01-0.7; Cr 1-1000; Cu 2-100; Ni 5-500; Zn 10-300; Pb 2-200 $mg \cdot kg^{-1}$.

On the other hand, human activities (industrial activities, power generation, agricultural activities, transport and atmospheric deposition) are commonly considered to be the reasons for metal increase in the environment when compared to natural sources. So, Campbell et al., (1983) compared the emitted amounts of heavy metals in atmosphere, and found out that Cd was emitted 15 times higher, Pb 100 times, Cu 13, and Zn 21 times higher from human activity than from natural processes. In the soils of Europe, the most contaminants are considered heavy metals and mineral oil, and approximately three million sites are estimated to have been potentially affected, and approximately 250,000 sites may need urgent remediation (Science Communication Unit, 2013).

The mass balance of metals (M) in soils can be calculated as follows (Alloway, 1995; Lombi and Gerzabek , 1998, Wuana and Okieimen, 2011):

$$M_{total} = (M_p + M_a + M_f + M_{ag} + M_{ow} + M_{ip}) - (M_{cr} + M_l)$$

where the indices p, a, f, ag, ow, ip, cr, and l indicate the parent material, the atmospheric deposition, the fertilizer sources, the agrochemical sources, the organic waste sources, other inorganic pollutants, crop removal, the losses by leaching and volatilization, respectively. Fig. 1.1 shows the schematic description of main sources of heavy metals in agricultural soil of Kosovo.

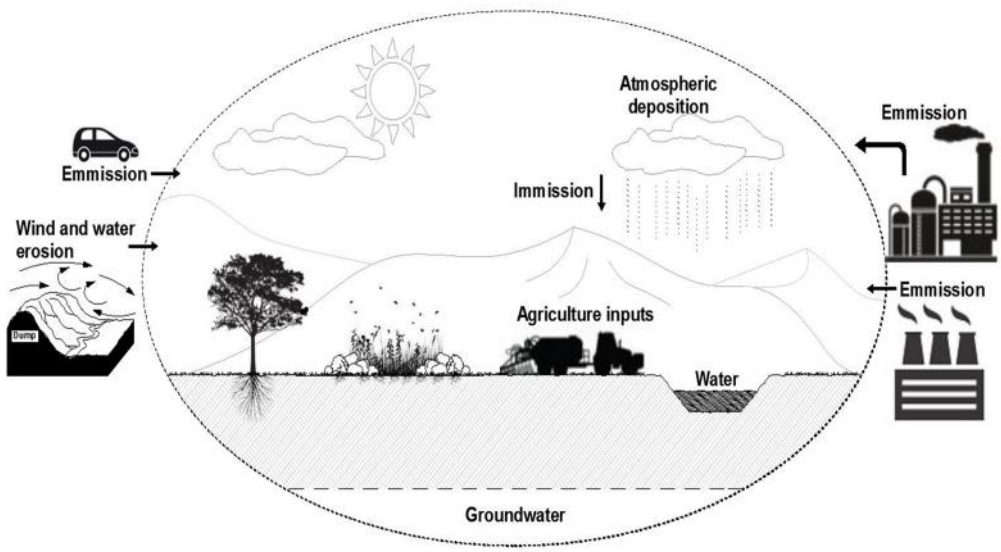


Figure 1. 1 Main sources of heavy metals in the agricultural soils of Kosovo

Kabata-Pendias (2011) reported that the input–output balance of metals in soils is likely to increase on a global scale with metal concentrations in surface soil, among growing industrial and agricultural activities. The balance of trace metals in agricultural soils varies greatly, depending on farm types, and crop farms may generate higher input of metals than animal farms (Kabata-Pendias, 2011).

1.1.3 Metal mobility and bioavailability

Soil is the main source of micronutrients for plants, as well as of essential and nonessential metals. The mobility and bioavailability of these elements can be a risk for the environment and plant uptake, even more for human health. The characterization of mobility and bioavailability of metals still isn't clearly defined and it is still part of many discussions. However, the mobile and bioavailable forms of metals are often defined as the forms of metals extractable in a chemical reagent based on correlation with the total metal uptake by plants (Black, 2010; Fairbrother et al., 2007; Chojnacka et al., 2005; Allen et al., 2002; McLaughlin et al., 2000; Alloway et al., 1988; McBride et al., 1997). Many different chemical reagents have been used to estimate the available form of metals. Synthetic chelating agents such as EDTA and DTPA have been often used to estimate potential bioavailability (Ivezić, 2011) and neutral salts (NH_4NO_3, $MgCl_2$, $CaCl_2$) to estimate mobile forms of metals.

Metals originating from various sources may finally reach the surface soil, and their behavior is influenced by their particular origin. The metal mobility depends firstly on weathering processes, and secondly on the anion (AEC) and cation exchange capacity (CEC) (Kabata-Pendias, 2004). The pedogenic processes influence the distribution and speciation of metals. The chemical speciation of metals controls their mobility and bioavailability through fixation by clay minerals and binding/complexing by soil organic matter (SOM) (Ivezić, 2012; Almås et al, 2007, Shuman, 1991). Additionally, pH, Eh, redox potential, clay content, organic matter content and nutrient balance can play an important role on behavior and plant uptake of metals (Kabata-Pendias, 2011, 2004; Kadovic et al., 2011; Jung, 2008; Smical et al, 2008; Lombi and Gerzabek, 1998). In many publications on this topic, soil pH and clay content are listed as the major soil factors controlling both the total and the relative uptake of Cd (Kabata-Pendias, 2011). Metal solubility tends to increase at lower pH and decrease at higher pH values. In well aerated (oxidizing) acid soils, Cd is mobile and available to plants, while in poorly aerated (reducing) neutral or alkaline soils, is less available (Kabata-Pendias, 2004). Bingham et al. (1980) found that the Cd content in rice grain is highly dependent upon soil pH, and it is highest at pH 5.5. The ability of the clays to bind the metal ions is correlated with their cation and anion exchange capacity (CEC, AEC) (Kabata-Pendias, 2011). Eriksson (1989) found that Cd was more soluble and plant available in sandy soil than in clay soil for a given total Cd concentration.

1.1.4. Plant metal uptake and prediction model

The soil – plant transfer of metals is a part of the chemical element cycle in nature (Kabata-Pendias, 2004). Metals are taken up by plants through the soil solution, and high concentration of metals in plants is a result of higher heavy metal contents in soil solution. Regarding the contamination and potential toxicity or ecotoxicity for normal growth of organisms, some of the metals (Zn, Cu, Mn, Mo, Ni) are essential at low concentrations. For that reason, concerning the toxicity and deficiency, heavy metals have a great importance for agricultural production and humankind through food chain. Concentrations of metals in soil solution are closely correlated with their mobility and bioavailability (Kabata-Pendias and Mukherjee, 2007). Metals in soil solution are mobile and may be easily taken up by plants and soil organisms. Also, these metals can be easily leached and thus influence the contamination of groundwater and surface water. Therefore, the chemistry of soil solution provides useful information on mobility and availability of metals and their transfer to the soil solution. Soil properties (pH, Eh, redox potential, clay content, organic matter content, cation exchange capacity, nutrient balance) play a crucial role in the relationship and transfer of metals between solid and soil solution through the precipitation-dissolution, adsorption-desorption, complexation-dissociation, and oxidation-reduction processes (He et al., 2005). Concerning the metal uptake, all plants can be divided into three groups: accumulators, indicators and excluders. Accumulator plants can grow in a medium with high content of metals as a result of the biodegradable or biotransformable contaminants in inert forms into their tissues. Generally, the content of metals in aerial plant tissues is greater than in soil. Indicator plants accumulate metals in their biomass at levels of the metal concentration in the soil. However, the excluder plants restrict contaminant uptake into their biomass, and maintain lower contaminant levels in their aerial tissues compared to soil concentrations (Leitenmaier and Küpper, 2013; Mehes-Smith et al., 2013; Tangahu et al., 2011; Mganga et al., 2011; Baker and Walker, 1990). Most of the accumulated metals remain in the roots. So for example, Zhang et al. (2000), found Cd content 10 times higher in the roots than in the shoots of 16 different wheat genotypes. In corn (Vojtechová and Leblová, 1991) and sunflower plants (Kastori et al. 1998; Simon, 1998) both Pb and Cd are accumulated more in the roots than in the shoots. That other metals (Zn, Cu, Mn and Fe) are accumulated more in roots is reported by Nouri et al. (2009). The accumulation of metals in plants in agricultural soils, especially in edible parts, is a great concern regarding potential threats (Li, 2011; Li 2006; Chien, 2008), and has urged a lot of prediction models to be developed in order to predict plant metal uptake (Li, 2011; Ge, 2000). To predict concentrations of metals in plants due to their uptake from soil or soil solution, mechanistic, empiric and mathematical models are used (Sterckeman, 2004; Adhikari, 2000; Sadana, 2000; Rengel, 1993; Gupta and Aten, 1993). Many authors, who have used these models, have been limited to specific contaminated sites or single species (Zarcinas, 2004; Kuo, 2004). A few investigations were made to describe metal uptake by rice, wheat and barley grain, based on metal and soil properties (Zhang, 2011; Adamsa, 2004). The relationship between metal concentrations in various soil extracts and plants is often described by a transfer factor. Krauss et al. (2002) used Freundlich – type functions to predict Cd, Cu, Pb and Zn concentrations in wheat grain and leaf. Multiple regression analysis was used successfully by Ivezić (2011) to predict metal concentrations in wheat grain in uncontaminated soil. Additionally, Neuhauser et al. (1995) and Sample et al. (1998a) have obtained significant regressions for the uptake of inorganic elements by earthworms using log-transformed soil and plant concentrations relationship.

1.2 Objectives

The main objective of this doctoral thesis is to assess the metal pollution in soils of Kosovo and to determine soil properties that control the potential bioavailability of these metals in soil. On this basis, a regression model predicting bioavailable metal concentrations and predicting plant uptake was developed. The more specific aims are:

- Determination of the pseudo-total level of heavy metals in agricultural soils of Kosovo (Chapter 2).
- Assessment of the potential bioavailability and mobile form of metals and assessment of the influence of soil properties on their bioavailability (Chapter 2).
- Determination of metal concentrations in different plants and transfer factors (Chapter 3).
- Testing the differences of metal concentrations in soil and plants between regions (Chapter 3).
- Development of regression models to predict plant metal uptake using soil properties (Chapter 3).

1.3 Materials and methods

1.3.1 Study area

In the first stage, the study area represents all agricultural soils in different regions of Kosovo, while in the second one we focused on agricultural soils of two regions, Mitrovica and Drenas, which are considered the most polluted regions of Kosovo. Kosovo is a country (10.877 km^2) in the center of the Balkan Peninsula (N 43° 16' – 41° 53' and E 21° 16 – 19° 59'). The entire region is divided into three zones developed in the Oligo-Miocene (Gashi and Spaho 2002): (i) two plains, the Dukagjini plain in the western, and the Kosovo plain in the eastern part, and (ii) adjacent hilly areas divided by rivers mainly originating in the (iii) surrounding mountain areas. The elevation ranges from 265 m to 2656 m above sea level, with about 80% of the entire area below 1.000 m. The climate in Kosovo is continental with Mediterranean influence in the west, with warm summers and cold winters. Air

temperatures range from –20°C to +35 °C. The main annual rainfall is about 650 mm, and about 170-200 days per year are frost-free. In the western part of Kosovo the climate is more moist (annual rainfall: about 800 mm) and warmer (196-225 frost-free days) than in the eastern part (Mehmeti et al. 2009, 2010; Elezaj and Kodra 2008).
Mitrovica region, which is located in the northern part of Kosovo was considered one of the main industrial sites of Former Yugoslavia and one of the most important mining districts in Europe (Nannoni et al., 2011). Within Mitrovica Industrial Park (Trepca) approximatly 40 mines, various concentrators, flotation and smelting plants, and several factories are included. This area has begun to be exploited intensively for the production of Pb, Zn, Au, Ag and Bi since the 1930s. The main industrial plants were the Zvecan smelter and the Trepca battery factory, being located near agricultural and residential areas, which have produced large amounts of elements and created large volumes of waste accumulated in enormous dumps close to the plants. In 1989, it was estimated that the Zvecan smelter emitted 730 t / year of particulate matter, as well as 438, 83, 3.6 t / year of Pb, Zn, and Cd, respectively (Frese et al., 2004). The agricultural land is developed along the valleys of Sitnica and Ibri rivers, and the main cultivated crops are wheat, maize, potato and some vegetables.

The Drenas region lies in the central part of Kosovo. The main industry in this part is Ferronikeli factory. The smelter of Ferronikel in Drenas, which is very close to agricultural and residential areas, started work in 1984, and was projected for processing 1 374 000 t of ore a year and the production of 52 000 t of iron-nickel (Haxhiaj and Haxhiaj, 2012). After decades of operation, around 3 millions of tons of granulated slag are stored in dump close to the factory. This slag is very light material with a low density (Veliu et al., 2009) and this material might be spread around by wind or water erosion.

1.3.2 Soil and Plant Sampling

In the first stage, 127 topsoil samples (0-30 cm depth in arable land and 0-5 cm in meadow) were collected throughout the agricultural area of Kosovo (Figure 2.1-

Chapter 2). In the second phase, 60 topsoil samples from the two above mentioned regions, were taken according to the random method (Manual 2006; Theocharopoulos et al. 2001; BBodSchV 1999). Each soil sampling was prepared out of 10 sub samples that had been taken with a distance of 10-50 m between them (depending on size and shape of the plots) using a hand auger. During the second phase, at the same time and at the same places, plant samples were also collected, that is, 20 wheat, 20 maize, 10 potato and 10 grass samples. All plant samples have been collected shortly before harvest.

1.3.3 Sample analysis

Dried and ground soil and plant samples were analyzed by standard methods. In general, soil pH, organic matter (OM), total carbon (C), nitrogen and particle size distribution was determined. Also, the pseudo-total contents of heavy metals, the exchangeable and mobile fractions of heavy metals in soil (potentially plant available and easily leachable) were determined. Further, the total content of metals in plants was extracted. All metals in soil and plant extracts were measured by an inductively coupled plasma optical emission spectrometer (ICP-OES), or an atomic absorption spectrometer (AAS). Quality assurance of extraction methods for soil and plant samples was provided by analyzing certified reference materials (CRMs). For more details see chapter 2 and 3.

1.3.4 Statistical analysis

The SPSS 22.0 software packages for Windows and Minitab (Statistical Software version 16) were using for statistical analysis. Different statistical analysis such as: descriptive statistics, analysis of variance, correlation, Student T-test, multiple linear regression analysis, etc., were performed (chapter 2 and 3).

1.4 Results and discussion

1.4.1 Spatial distribution of metals in agricultural soils of Kosovo

Metal content and bioavailability of these metals can be a risk for the environment and plant uptake, and moreover for human health. Kosovo has a lack and no accurate data about the level of heavy metals in agricultural soils and contaminated surface soils. For this reason, the first step of this study was to determine the total level of metals in agricultural soils of Kosovo, and to assess the bioavailability of these metals. To prove this aim, a large set of soil samples were taken from agricultural soils of Kosovo. Soil samples were analyzed for the following parameters: pH, organic matter and heavy metals (pseudo total, potential bioavailability and mobile forms Cd, Cr, Cu, Ni, Pb and Zn) (Chapter 2). Descriptive statistics of all soil properties are given in Table 2.2. The content of metals in some sampling sites (mainly around the contamination sources) was very high and most of elements (Ni, Zn, Cu, Cr, Cd, Pb) show a wide range of concentrations, and their mean levels reach 1.5 to 2.5 times of those of their median values. Therefore, the content of Ni in 5% of analyzed samples showed a very high concentration over 400 mg kg^{-1}, whereas 62% of samples exceeded the critical limit set by the EU standards (75 mg kg^{-1}, EC 1986) (Figure 2.2). On the other hand, the bioavailability and mobile forms of Ni were very low with the mean of 8.05 and 0.51 mg kg^{-1}, respectively (Table 2.2). According to BBodSchV (1999) (1.5 mg kg^{-1}), only 6% of all investigated samples exceeded the limit values for Ni extracted by ammonium nitrate. These sites were mainly close to contaminated sources ("Ferronikeli" factory). This low mobile form of Ni can be explained by the fact that it has geological origin of parent material in unpolluted agricultural soils of Kosovo. However, 9% of the analyzed samples for Pb surpassed the critical limit concerning the EU standards (300 mg kg^{-1}, EC 1986). The potential bioavailability (extracted by EDTA) and mobile forms (extracted by ammonium nitrate) of Pb in soils were very high with a mean 41.2 and 0.11 mg kg^{-1}, respectively. Regarding permitted values for Pb extracted by ammonium nitrate in Germany (BBodSchV, 1999) (0.1 mg kg^{-1}), 29% of samples exceeded that limit. The range concerning the extractability of the different metals by EDTA resulted as follows: Cu ≈ Pb ≈ Cd >> Ni ≈ Zn >> Cr, while the order of metals extracted by NH_4NO_3 wasas follows: Cd >>Ni>Pb>Cu>Zn>>Cr (Figure 2.4). A high correlation of pseudo-total concentration was shown among Ni and Cr (R^2=0.773) and Zn and Pb (R^2=0.7554) (Figure 2.3). Cultivation of agricultural plants in these areas with a high mobility of Pb can be a risk for human health. The regression analysis has shown that the pseudo-total metal content significantly influenced ($p<0.001$) the amount of metals extracted with EDTA. The value of pH and the content of organic matter did not show any significance with regard to EDTA extractable metals (Table 2.3.).

A high significance ($p<0.001$) between soil pH value and mobile Ni, Zn, Cd and Pb has been shown. Correlation of mobile fractions contents with potential bioavailable metal did show significance ($p<0.001$) for Ni, Zn and Cd, as well as for Cr on a significance level of $p<0.05$. The pseudo-total metal content was significant ($p<0.001$) only for mobile Ni (Table 2.4.).

1.4.2 Plant metal uptake and transfer factors for two contaminated regions of Kosovo

1.4.2.1 Pseudo total content of metals and their fractions in agricultural soils

Increasing of metal concentrations in top soil (especially near to industry) and during uptake by plants may eventually threat human health through the food chain. Therefore, another important aim of this study was to determine the concentration and bioavailability of metals in soils of two contaminated regions of Kosovo, to describe their transfer into different plants, which are commonly cultivated in these regions, and to assess the influence of soil parameters (Chapter 3). Pseudo total content of metals is given as box-plots (Figure 3.1). Increased mean values compared to the median values indicate a heterogeneous distribution of the contamination with Cd, Cr, Ni, Pb and Zn. Content of Cd showed an increase up to 10.3 $mg \cdot kg^{-1}$ and Pb and Zn show severely increased concentrations at some sampling sites (up to 1450 and 1700 $mg \cdot kg^{-1}$ respectively) in Mitrovica region. For the region of Drenas we found some contaminated sites with Cr and Ni which may be a result of the emissions of the factory “Ferronikel”. Contamination with Cr and Ni on these sites increased up to 626 and 1560 $mg \cdot kg^{-1}$ respectively.

Student T-test showed significant differences between regions for all investigated elements on a significance level of $p<0.001$, except for Ni concentrations which were different on a significance level of $p<0.05$.

The potential plant bioavailability (EDTA extractable) of metals (as a fraction of EDTA extractable metal by *aqua regia* extractable metals) showed high variation between metals. So, Cd showed the highest potential bioavailability, from approx. 50% to more than 80%, in Drenas region, and from 35% to more than 70% in Mitrovica region. These results confirm the findings of Asami (1984) who reported that half or more of the total Cd in Japanese paddy soil is exchangeable and available to plants. The lowest extraction efficiency was encountered for Cr, from 0.02 to 0.13%, with a mean of 0.05, in Drenas region, and from 0.02 to 0.08% (mean 0.06) in Mitrovica region (Figure 3.2). That Cr occurs in soils mainly (>80% of total content) in the immobile residual fraction and is hardly mobile, thus not easily available to plants, was shown by Kabata-Pendias (2011), Hooda (2010), Kabata-Pendias and Mukherjee (2007). The low EDTA – extractability of Cr is confirmed in other studies (*Megremi*, 2009; *Cappuyns*, 2012).

In addition, Student t-test showed high significant differences of EDTA extractable metal concentrations between regions, except for the Ni concentrations which did not differ between the two regions. All other elements showed differences between regions on a high significance level ($p<0.001$).

The mobile form (Ammonium nitrate extractable) of heavy metals (as a fraction of ammonium nitrate extractable metals by *aqua regia* extractable metals), showed the similar trend behavior as plant bioavailability form (PBF). Cd showed highest mobility, from 0.25 to more than 25%, in Drenas region, and from 0.4 to approx. 20% in Mitrovica region. The lowest extraction efficiency was encountered for Cr, up to 0.006%, in Mitrovica region, and up to 0.009% in Drenas region (Figure 3.3.). Extractability by NH_4NO_3 follows this order in Drenas region: Cd > Ni > Cu > Zn > Pb > Cr, and in Mitrovica region: Cd > Zn > Ni > Cu > Pb >> Cr. Student T-test showed high significant differences of mobile form (MF) concentrations in soils between regions for Cd ($p<0.001$), high significant differences for Zn ($p<0.01$) and significant differences for Cr and Pb ($p<0.05$), whereas Cu and Ni concentrations did not differ between the two regions.

1.4.2.2 Metal content in plants and transfer factor

In Table 3.2 it is shown the content of metals in different plants. The concentrations of Cd, Cr, Ni, and Pb in wheat were higher in vegetative than in generative parts in both regions. However, Cu and Zn were higher in generative than in vegetative parts in wheat, similar to data previously reported by *Puschenreiter* and *Horak* (2000). In corn, all metal concentrations were higher in vegetative than in generative parts. In general, metal concentrations in the analyzed plants showed comparable values with the reported ones by several European countries (*Kabata-Pendias*, 2011), except for Pb in potato tubers, which exceeded the limit of the permitted level for Pb content in foodstuff regarding EU standard (Commission Regulation (EC) No 1881/2006). In addition, some potato samples showed concentrations of Cd above the permitted level. On the one hand, this indicates a risk due to metal transfer into food. On the other, EC 1881/2006 is targeting on peeled potato, while we analyzed it unpeeled. Therefore, further assessment should be done to deny or approve this possible risk.

The transfer of metals from soil to plant (transfer factor-TF) was used to determine the relative uptake of heavy metals by plants (calculated as divided of metal contents in plant by EDTA extractable metal in soil) (table 3.3). According to *Kabata-Pendias* (2011), Cr is poorly soluble in soil solution and not easily taken up by plants; Pb is relatively strongly sorbed by soil particles and not readily transported to above-ground parts of plants; Cu, Ni are mobile in soil and readily taken up by plants; Cd, Zn are very mobile in soil and easily bioaccumulated by plants and our results do confirm this argument. In general, TF was higher in soil samples with low content of metals, compared to soil samples with high content of metals, which confirmed the previous finding by *Lübben* (1993, cited by *Puschenreiter* and *Horak*, 2000). Therefore, we suggest being careful when using this factor in the future, as it seems to depend on the concentration level in soil. This means, from contaminated soils nonessential metals may be taken up with a relatively small TF, but in absolute numbers the concentration of the plant would be high and therefore can be dangerous for human health.

1.4.2.3 Model to predict metal content in plants

The model for multiple linear regressions, given n observations, is

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + ... + \beta_p x_p + \varepsilon$$

where error term ε is uncorrelated with $x_1, x_2, ..., x_p$ and is distributed with mean zero and variance σ^2.

Generally, metal extraction by EDTA can be indicative for concentration of metals in plants. In wheat plants (for both vegetative and generative parts) Cd, Pb and Zn concentrations could be explained highly significant ($p < 0.001$) by the chosen parameters with R^2 values higher than 0.7. For the other metals analyzed, this model did not show significant correlation and was not able to explain plant concentration (Table 3.4.). Our results are in line with the reported results from *Krauss* et al. (2002) and *Ivezić* (2011).

In the prediction of metal contents in corn, both in vegetative and generative parts, exclusively metal EDTA extraction plays a significant role (R^2 values from 0.22 to 0.88). So, prediction model for Cd, Pb, Zn in plant and Zn in grain showed high significance ($p<0.001$). For Ni in grain the significance level was 99% ($p<0.01$), and for Cd, Pb in grain the significance level was 95% ($p<0.05$). The prediction of Cr in plant was not significant (Table 3.4.).

The prediction model for metal concentration in potato tubers showed high significance ($p<0.001$) for Pb and Zn (R^2 values from 0.92 to 0.97), significance ($p<0.01$) for Cd (R^2: 0.84), and significance ($p<0.05$) for Cu (R^2: 0.54). Cr and Ni didn't show any significance at level 95% (Table 3.4.).

Concerning the grass plant metal content, the model explained significantly Ni at level 99% and Cr and Zn at level 95% (Table 3.4.).

Regarding the relative explanation index (REI) of variance, Pb and Zn-EDTA extract showed higher contribution in REI with 91.4 respectively 92.7 and the lowest contribution was for pH 5.3% in potato tubers (Figure 3.4c.). Further explanations of REI are given in Figure 3.4.

1.5 Conclusions and recommendations

Based on the results of the investigations in agricultural soils of Kosovo, the following brief conclusions can be reported:

The pseudo total content of Cd, Pb, Zn, Ni and Cr in some soil samples of the under investigated agricultural soils of Kosovo were significantly higher than the target values for these metals according to both national, EU and Netherland standards (Administrative Project, 2009; Council directive 86/278/EEC, 1986; the New Dutch List, 2000).

High concentrations of heavy metals have been noticed near contamination sources (mainly near metals' industries), which, in some cases, have exceeded many times the allowed limits. Moreover, the potential bioavailable form of metals around these sources has shown very high values, presenting a serious hazard for human health, since these soils are being cultivated and their products being consumed.

The comparison between the two regions of all Cd, Pb and Zn forms, revealed that the metal concentrations were significantly higher in Mitrovica region. The concentration of Cd in potato tubers in Mitrovica region indicates a risk due to metal transfer into food, where high amounts of it were also found in wheat grain. Further, Pb was found in high amounts in potato tubers and corn grain, but not detected in wheat grain in the same region.

The regression analysis has shown that the pseudo-total metal content significantly influenced ($p<0.001$) the amount of metals extracted with EDTA. The content of organic matter and the value of pH did not show any significance in the amount of extracted metals with EDTA.

Strong correlation ($p<0.001$) between soil pH value and mobile Ni, Zn, Cd and Pb has been shown. Correlation of potential bioavailable metal contents with mobile fractions did show significance ($p<0.001$) for Ni, Zn and Cd, as well as for Cr on a significance level of $p<0.05$. The pseudo-total metal content was significant ($p<0.001$) only for mobile Ni.

Even though bioavailability of metals was high, the TF (as ratio of metal concentrations in plant to EDTA-extractable) from soil to plant was higher for micronutrients (Zn and Cu) than nonessential metals (Cd and Pb). Further, TF was higher in vegetative than generative parts for toxic metals and conversely for micronutrients. So, we suggest being careful when using this parameter any longer, because it seems not suitable to assess a risk for plant cultivation. In our investigation, some plants with high concentrations of toxic metals showed lower TF compared to other samples of the same plant species with lower content of toxic metals.

The multiple regression analysis was a good model to predict Cd, Pb and Zn concentrations in wheat, corn, and potatoes. However, one should be careful when interpreting the model results, as the R^2 values for some metal – plant combinations are rather low (i.e. below 0.5 which means that only 50% of variance could be explained). Soil pH played a significant role for the uptake of Cd and Zn in wheat and potato plants, and the control of soil pH is a key to control the availability of these metals. Additionally, clay content was a significant parameter influencing the concentration of Cd in wheat and potato plants, while the C content of the soil was significant for Zn in wheat and grass plants, as well as for Cd in grass plants.

Concerning human health protection, and based on the results of investigated soil and plant samples, we can address some suggestions and recommendations for managing the agricultural soil in the future:

Because the pH has an important role in mobility and bioavailability of metals, it is regularly required the application of calcification on soil, using lime or other calcic materials and permanently monitoring soil pH, especially in acidic soils and near the contaminant source.

It is required to constantly investigate heavy metal concentrations in agricultural products, especially those which are cultivated close to contamination sources. In Mitrovica region, it is recommended not to cultivate vegetables, especially leafy ones like lettuce, cabbage, spinach, since it is well known that they have the ability to accumulate metals. However, wheat and corn can continue to be cultivated, since we have not found high concentrations of metals in these plants, especially in grain/consumption parts.

Contaminated sites with metals may also be associated with organic pollutants, so it is very important to investigate them in the future.

And from the competent authority, who manages agricultural soils, it is required to think about remediation in the future.

2

Spatial distribution of heavy metals and assessment of their bioavailability in agricultural soils of Kosovo

Muhamet ZOGAJ, Musaj PAÇARIZI, Rolf-Alexander DÜRING, 2014. Spatial distribution of heavy metals and assessment of their bioavailability in agricultural soils of Kosovo, Carpathian Journal of Earth and Environmental Sciences, Vol. 9, No. 1, p. 221 - 230

Abstract:

From topsoil, 127 samples were collected in agricultural areas of Kosovo, which were analyzed for heavy metals (HM), by extraction with aqua-regia (pseudototal concentration), NH_4OAc-EDTA (potential bioavailable) and NH_4NO_3 (mobile fraction). 62% of the soil samples showed elevated Ni concentrations, whereas increased values for Pb, Cd, Zn and Cr were in 9%, 6%, 5%, and 2% of the sample set, respectively. Cu was below threshold values in all analyzed samples. In order to assess the bioavailability of heavy metals, relevant soil parameters were determined. Regarding mobile fractions of HM, only Ni was significantly influenced by its total concentrations. For most of HM in mobile fractions, soil pH significantly impacted the extracted metal amounts. On some field sites crop production may be under risk due to elevated metal concentrations in not only the pseudototal but also in the potentially bioavailable and mobile fractions.

Keywords: pseudototal concentrations, assessment of bioavailable fraction, mobile fraction

2.1 Introduction

Mineral dispersion and human activity are two main sources impacting the soil-plant system with heavy metals. Campbell et al., (1983) compared the emitted amounts of heavy metals in the atmosphere, and found that 15 times higher Cd, 100 times higher Pb, 13 times higher Cu, and 21 times higher Zn is emitted from human activity than from natural processes. Thence, soil contamination by heavy elements represents a worldwide environmental concern over decades. These elements can be transferred to the hydrosphere and biosphere, thereby posing a hazard to human health. Consequently, the mobility and bioavailability of heavy elements in soil play an important role in the uptake of these contaminants by vegetation and animals (Nanoni et al., 2011). Total concentrations of trace metals in soil are poor indicators for their bioavailability, yet they are commonly used for the determination of maximum permissible levels in the legislation of many countries. It is of great importance to estimate the total contents of toxic elements and their speciation in soils (Chakroun et al., 2013). Water soluble and exchangeable forms of heavy metals are considered readily mobile and available to plants. Several authors have examined the controlling parameters influencing metal solubility in soils and expressed the correlations by regression models (Ivezić et al., 2012; Gandois et al., 2010; Groenenberg et al., 2010). Such regression models usually include total concentration of trace metals (M (tot)), soil pH, soil organic matter (SOM) and clay content. With increasing pH, content of organic matter, and clay the bioavailability of most metals decreases due to their increased adsorption (Takac et al., 2009).

Several reagents (e.g., $CaCl_2$, NH_4OAc, NH_4NO_3, complexing agents) have been used to extract the ”mobile” or “bioavailable” forms of heavy metals with single extraction procedures (Narwal et al., 1999; He & Singh 1995, Damian et al., 2008). Single extractions provide information on potential mobility as well as bioavailability and plant uptake of heavy metals (Singh 1997).

Several studies showed that the mining discharges, including the ore processing industry, represent a potential source of contamination for soil (Ditoiu & Osean 2007, Lăcătușu et al., 2009, Galfati et al., 2011, Chakroun et al., 2013). The main heavy metal source contaminating the environment and agricultural soils in Kosovo is the industry, which is mostly located in the eastern part of the country. The following heavy industry sites are found in this area: the ore-metallurgic combine “Trepça” in Mitrovica, the Kosovo Energetic Corporation in Obiliq, “Ferronikeli” in Drenas, the Battery Factory Ni-Cd “IBG-Gjilan”, „Cementorja“ Hani i Elezit. Many authors report high levels of heavy metals in agricultural soils which are located near these contamination sources, and which surpass many times the permitted values for soils (Nanoni et al., 2011; Maxhuni et al., 2011; Borgna et al., 2009; Elezi & Jusufi 1996). Nevertheless, up to now, there are no accurate data in Kosovo about the level of heavy metals in agricultural soils and contaminated surface soils. Therefore, the aim of this paper is to determine the total level of heavy metals in agricultural soils of Kosovo, to assess the bioavailability of the metals, and to assess the influence of pH and organic matter on their potential for plant uptake.

2.2 Materials and methods

2.2.1 Study area

The study area represents agricultural soils in different regions of Kosovo. The Kosovo is a country (10.877 km^2) in the center of the Balkan Peninsula (N 43° 16' – 41° 53' and E 21° 16 – 19° 59'). The entire region is divided into three zones developed in the Oligo-Miocene (Gashi & Spaho 2002): (i) two plains, the Dukagjini plain in the western, and the Kosovo plain in the eastern part, and (ii) adjacent hilly areas divided by rivers mainly originating in the (iii) surrounding mountain areas. The elevation ranges from 265 m to 2656 m above sea level, with about 80% of the entire area below 1.000 m. The climate in Kosovo is Continental with Mediterranean influence in the west, with warm summers and cold winters. Air temperatures range from –20°C to +35°C. The main annual rainfall is about 650 mm, and about 170-200 days per year are frost-free. In the western part of Kosovo the climate is more moist (annual rainfall: about 800 mm) and warmer (196-225 frost-free days) than in the eastern part (Mehmeti et al., 2009, 2010; Elezaj & Kodra 2008).

According to a digital map of soil types (scale 1 : 50000) provided by the Chairman of Soil Sciences of Prishtina University (Elezi et al., 2004) and referring to the WRB-soil classification (IUSS Working Group WRB 2006), more than 80% of agricultural soil are cambisols, vertisols, fluvisols, regosols and gleysols.

2.2.2 Soil Sampling

In total, 127 topsoil samples (0-30 cm depth in arable land and 0-5 cm in meadow) were collected throughout the agricultural area of Kosovo (Fig. 2.1). Each soil sampling has been prepared out of 5 sub samples that have been taken with a distance of 10-50 m between them using a hand auger. The topsoil samples were collected according to the random method (Bundes Bodenschutz und Altlastenverordnung (BbodSchV) 1999; Manual 2006; Theocharopoulos et al., 2011 a, b). A larger number of samples per surface unit has been taken near the contamination sources (smelting, mining, power plant, etc.), whereas a smaller number has been taken from areas that are further remote from those sources.

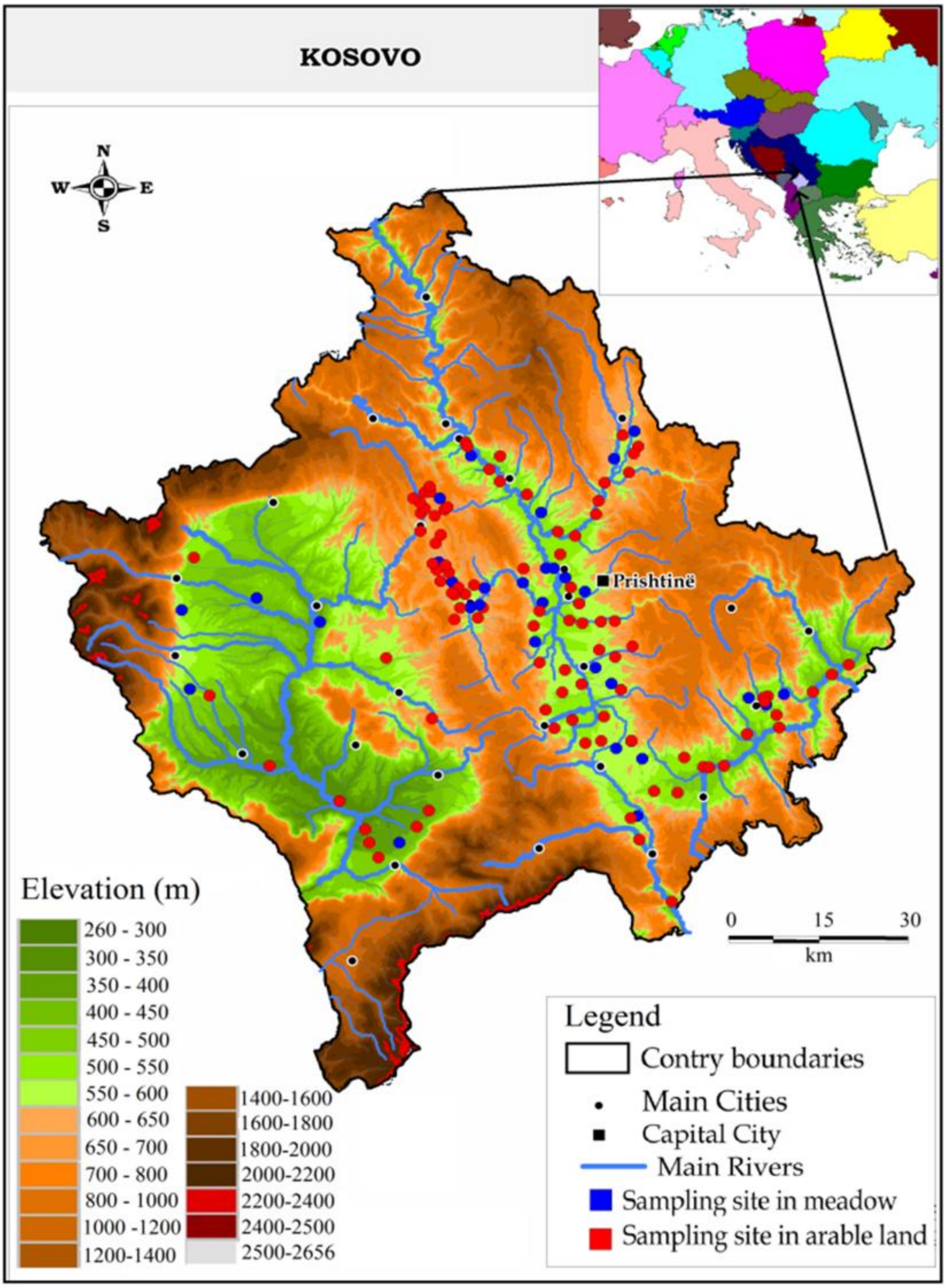


Figure 2. 1 Soil sampling sites in the study area.

Table 2. 1 Correlation of heavy metal concentrations (mg kg^{-1}) from composite soil sampling and single soil sampling (average)

	Cd		Cr		Cu		Ni		Pb		Zn	
No	CSS	ASS	CSS	ASS	CSS	ASS	CSS	ASS	CSS	ASS	CSS	ASS
1	0.266	0.282	251.66	246.30	71.67	71.03	415.90	420.72	33.24	32.65	99.85	99.80
2	0.056	0.044	52.08	60.47	21.99	23.20	33.01	36.49	30.84	31.95	39.72	42.14
3	0.113	0.095	201.77	199.95	39.11	37.95	236.08	226.98	28.13	27.26	87.61	85.42
4	0.204	0.228	124.70	122.36	35.09	32.99	155.82	155.35	71.19	71.34	101.09	99.74
5	0.346	0.322	126.81	128.07	31.91	29.90	99.14	92.31	142.58	135.61	91.76	91.92
6	0.109	0.112	89.17	87.86	29.98	35.54	172.07	170.91	43.42	42.22	84.75	84.24
7	0.145	0.132	142.20	139.41	38.63	36.32	164.90	164.08	43.68	42.76	94.20	94.05
8	1.293	1.357	204.20	190.65	69.70	69.06	236.98	251.78	1183.75	1215.71	436.68	458.01
R^2	0.99		0.99		0.98		0.99		0.99		0.99	

CSS-composite soil sampling, SSS- single soil sampling (average)

For quality assurance and to prove representativeness of soil sampling, we took 8 samples that have been analyzed individually (8x5=40), and composite samples containing 5 sub samples. We could show that there is no significant difference between sub samples, but a very high correlation between the composite samples and the average of sub samples has been revealed (Table 2.1). A similar correlation between the average of sub samples and the composite ones, has also been reported by Zgorelec et al., (2011).

2.2.3 Sample analysis

Soil samples were dried at room temperature, sieved for 2 mm, partially finely ground, and stored at room temperature until analysis. Soil pH was measured in H_2O suspension of soil and 0.01M $CaCl_2$ with a ratio of 1:2.5 (DIN ISO 10390 2005). The total amount of organic matter (OM) was determined by the ignition method.

The pseudo-total contents of heavy metals in soil were extracted with *aqua regia* (3 parts of 35% HCl and 1 part of 65% HNO_3) from finely ground samples (DIN 11466 1995). The term pseudo-total stands for the extraction with *aqua regia*, which does not completely destroy silicates. As this pseudo- total content is insufficient to determine ecotoxicologically relevant heavy metals, the exchangeable and mobile fractions of heavy metals (potentially plant available and easily leachable), were also extracted with NH_4OAc-EDTA – extract (Ammonium acetate and

Ethylenediaminetetraacetic acid) and 1M NH_4NO_3 (DIN 19730 2009) according to the German law (BBodSchV 1999).

The pseudo-total contents of heavy metals in *aqua regia* extracts were measured with an atomic absorption spectrometer (AAS) (MSeries, Thermo, at the Faculty of Agricultural and Veterinary-University of Prishtina) by the flame method. For determination of exchangeable and mobile forms of metals inductively coupled plasma optical emission spectrometry (ICP-OES; Varian 720ES) at Justus Liebig University (JLU) was used due to its higher sensitivity compared to the AAS flame methodology.

For quality assurance certified reference materials ("soil 1" and "soil 2", test 2004, 2005) supplied by the "Centre for Agricultural Technology Augustenberg" (Karlsruhe) were used for measurement of heavy metals in *aqua regia* extract and internal reference material (JLU) was used for plant available and mobile forms of heavy metals. Also, 10% of total samples were additionally extracted and analyzed at JLU.

2.2.4 Data analysis

Descriptive statistics analysis and multiple regression analysis were performed using Minitab (Statistical Software version 16).

2.3 Results and discussion

Table 2 shows the main descriptive statistic indexes for 21 analyzed parameters (pH_{H20}, pH_{CaCl2}, OM, Ni_{AR}, Ni_{EDTA}, Ni_{AN}, Zn_{AR}, Zn_{EDTA}, Zn_{AN}, Cu_{AR}, CuEDTA, CuAN, Cr_{AR}, Cr_{EDTA}, Cr_{AN}, Cd_{AR}, Cd_{EDTA}, Cd_{AN}, Pb_{AR}, Pb_{EDTA}, Pb_{AN}) in agricultural soils of Kosovo.

Most of elements (Ni, Zn, Cu, Cr, Cd, Pb) show a wide range of concentrations, and their mean levels reach 1.5 to 2.5 times of those of their median values.

Table 2. 2 The main descriptive statistics of the analyzed parameters

Variable	Mean	SE Mean	StDev	Min	Q1	Median	Q3	Max
pH_{H20}	7.0674	0.0503	0.5666	5.66	6.56	7.09	7.61	8.02
pH_{CaCl2}	6.7261	0.0497	0.5596	5.42	6.25	6.87	7.2	7.78
OM(%)	6.794	0.205	2.309	2.472	5.085	6.541	8.381	15.918
$Ni_{AR\ (mg\ kg-1)}$	156.5	26	293	12.5	49.4	103.4	161.2	2864
$Ni_{EDTA\ (mg\ kg-1)}$	8.047	0.75	8.447	0.636	2.779	5.645	10.416	60.095
$Ni_{AN\ (mg\ kg-1)}$	0.512	0.105	1.179	0.013	0.06	0.13	0.442	9.865
$Zn_{AR\ (mg\ kg-1)}$	90	12.9	145.3	14.5	38.1	56	78.6	1284.1
$Zn_{EDTA\ (mg\ kg-1)}$	9.71	3.24	36.46	0.6	1.7	2.28	4.12	372.87
$Zn_{AN\ (mg\ kg-1)}$	0.2595	0.0522	0.5879	0.001	0.021	0.0627	0.1888	4.0397
$Cu_{AR\ (mg\ kg-1)}$	33.35	1.25	14.05	9.36	23.29	31.08	39.43	92.65
$Cu_{EDTA\ (mg\ kg-1)}$	5.806	0.326	3.671	1.511	3.568	5.133	6.73	26.61
$Cu_{AN\ (mg\ kg-1)}$	0.0449	0.0048	0.05415	<ND	0.0193	0.0346	0.0545	0.4602
$Cr_{AR\ (mg\ kg-1)}$	92.3	12.2	137.5	17.3	45.1	67.4	102.8	1444.7
$Cr_{EDTA\ (mg\ kg-1)}$	0.0799	0.0044	0.0492	0.0244	0.0467	0.0645	0.0974	0.2989
$Cr_{AN\ (mg\ kg-1)}$	0.0071	0.0003	0.0033	0.0022	0.0049	0.0067	0.0086	0.0236
$Cd_{AR\ (mg\ kg-1)}$	1.005	0.129	1.458	0.036	0.521	0.661	0.87	14.16
$Cd_{EDTA\ (mg\ kg-1)}$	0.2742	0.0762	0.8591	0.0292	0.0737	0.0999	0.1455	9.0367
$Cd_{AN\ (mg\ kg-1)}$	0.009	0.0021	0.0232	<ND	0.0009	0.0024	0.0071	0.2106
$Pb_{AR\ (mg\ kg-1)}$	163.3	30.1	338.7	15.6	43.6	60.1	92.5	2206.3
$Pb_{EDTA\ (mg\ kg-1)}$	41.2	10.5	118.4	1.8	6.8	10.1	13.9	870.3
$Pb_{AN\ (mg\ kg-1)}$	0.115	0.0228	0.2568	0.0089	0.052	0.0787	0.1058	2.8702

ND - Not detected, Mean - arithmetic mean of soil samples, SE Mean - Standard error of the mean, StDev - standard deviation, Min – minimum, Q1 – firs quartile, Median – median of soil samples, Q3 – third quartile, Max – maximum

2.3.1 Pseudo total contents for distinct heavy metals in agricultural soils

2.3.1.1 Lead

Pb concentrations vary from 15.6 to 2206.3 mg kg^{-1}, with a mean 163.3 mg kg^{-1} and a significantly lower median (60.1 mg kg^{-1}). 5% of analyzed samples showed a very high concentration over 1000 mg kg^{-1}, while 9% of samples surpassed the critical limit concerning the EU standards (300 mg kg^{-1}, EC 1986). Taking into account the permitted values in Kosovo (50 mg kg^{-1}, administrative instruction 2009), and Germany (100 mg kg^{-1}, BMU 2007, 1992; BBodSchV 1999), 67% respectively 23% of samples exceeded the permitted values (Fig. 2.2). Similar values on high levels of Pb in some areas of Kosovo, mainly around the contamination sources, have been reported also by other authors: For instance, Nannoni et al., (2011) determined high levels of Pb from 53.4 to 5536 mg kg^{-1}, Borgna et al. (2009) 49.9 to 37123 mg kg^{-1}, Elezi & Jusufi (1996) 235 to 7500 mg kg^{-1}. However, studies on agricultural areas situated far from contamination sources reported lower values for Pb. Thus, Maxhuni et al., (2011), report levels from 1.9 to 173.7 mg kg^{-1} of Pb in the root system zone, depending on the distance from roads.

2.3.1.2 Nickel

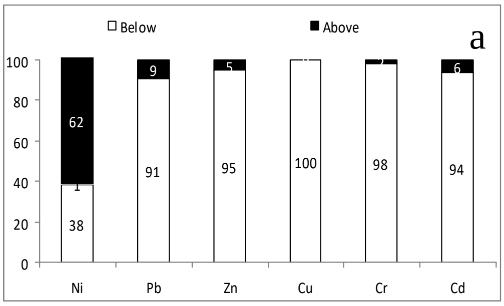
Concentrations of Ni range from 12.5 to 2864 mg kg^{-1}, with a mean of 156.5 mg kg^{-1} and a significantly lower median (103.4 mg kg^{-1}). 5% of analyzed samples showed a very high concentration over 400 mg kg^{-1}, whereas 62% of samples exceeded the critical limit set by the EU standards (75 mg kg^{-1}, EC 1986). Regarding the limit of permitted values in Kosovo and Germany (50 mg kg^{-1}, administrative instruction 2009; BMU 2007, 1992; BBodSchV 1999), 74% of samples exceeded that limit (Fig. 2.2). Similar values for high levels of Ni in some areas in Kosovo, mainly around the contamination sources, have also been reported by Borgna et al., (2009), who determined levels of Ni from 12.3 to 2842 mg kg^{-1}.

2.3.1.3 Zinc

The content of Zn varies from 14.5 to 1284.1 mg kg^{-1}, with a mean of 90.0 mg kg^{-1} and a significantly lower median (56.0 mg kg^{-1}) (Table 2.2). Only 5% of samples exceeded the critical limit as to EU standards and that of Kosovo (300 mg kg^{-1}, EC 1986; administrative instruction 2009). Compared to the limit values in Germany (200 mg kg^{-1}, BMU 2007, 1992; BBodSchV 1999), 8% of samples exceeded it (Fig. 2.2). Similar values of high levels of Zn in some areas in Kosovo, mainly around the contamination sources, have also been reported by Borgna et al., (2009) who determined levels of Zn from 31.7 to 17239 mg kg^{-1}. Similarly, Elezi & Jusufi (1996) reported values from 150 to 7000 mg kg^{-1}.

Metals	Threshold		
	EU	Kosovo	Germany
Ni	75	50	50
Pb	300	50	100
Zn	300	300	200
Cu	140	100	60
Cr	380*	50	100
Cd	1-3	2	1.5

*Dutch list



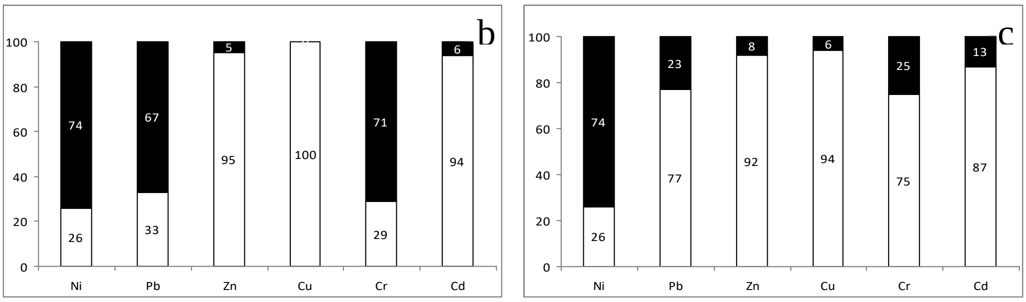


Figure 2. 2 Percentage of the total amount of heavy metals (mg kg^{-1}), which are under or above the acceptable levels, according to different countries, a) European Union, b) Kosovo, c) Germany.

2.3.1.4 Cadmium

The content of Cd also showed high variations from 0.036 to 14.16 mg kg^{-1}, with a mean of 1.005 mg kg^{-1} and median of 0.661 mg kg^{-1} (Table 2.2). Only 6% of samples exceeded the critical limit as to EU standards and Kosovo's standards (3 mg kg^{-1}, EC, 1986, 2 mg kg^{-1} administrative instruction, 2009). Regarding the legislation for soils in Germany (BMU 2007, 1992; BBodSchV 1999), 13% of samples exceeded the limit of 1.5 mg kg^{-1} (Fig. 2.2). Similar values on high levels of Cd in some areas in Kosovo, mainly around the contamination resources, have been reported by other authors as well. Nannoni et al., (2011) report the high level of Cd from 0.36 to 11.8 mg kg^{-1} and Borgna et al., (2009) from 0.37 to 69.7 mg kg^{-1}.

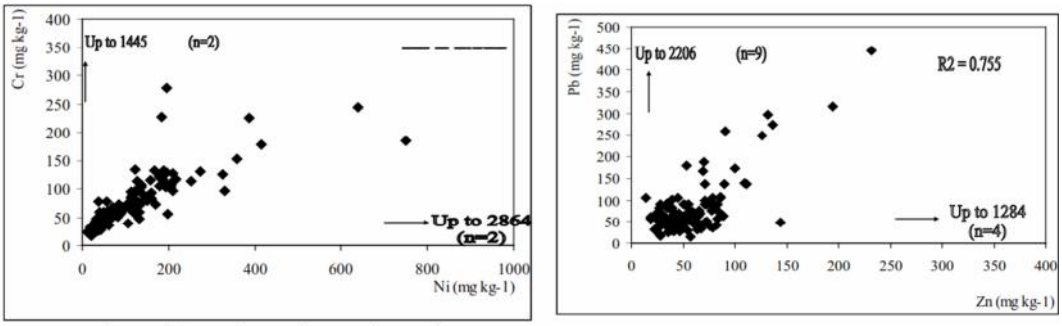
2.3.1.5 Copper

Concentration of Cu revealed variations from 9.36 to 92.65 mg kg^{-1}, with a mean of 33.35 mg kg^{-1} and a median of 31.08 mg kg^{-1} (Table 2.2). All analyzed samples have been below the critical limit regarding the EU standards and that of Kosovo (140 mg kg^{-1}, EC 1986; 100 mg kg^{-1}, administrative instruction 2009). Regarding permitted values in Germany (BMU 2007, 1992; BBodSchV 1999), 6% of samples were above the limit of 60 mg kg^{-1} (Fig. 2.2). Other authors also reported similar values of Cu levels concerning some areas in Kosovo. Nannoni et al., (2011) measured levels of Cu from 17.8 to 134 mg kg^{-1} and Borgna et al., (2009) from 2 to 563.4 mg kg^{-1}.

2.3.1.6 Chromium

In the analyzed samples, the content of Cr showed high variations from 17.3 to 1444.7 mg kg^{-1}, with a mean of 92.3 mg kg^{-1} and median 67.4 mg kg^{-1} (Table 2.2). Only 2% of samples exceeded the critical limit regarding the Dutch list (380 mg kg^{-1}, 1996). Concerning the permitted values in Kosovo (50 mg kg^{-1}, administrative instruction 2009), and Germany (100 mg kg^{-1}, BMU 2007, 1992; BBodSchV 1999), 71% respectively 25% of samples were above limit (Fig. 2.2). Similar values of high levels of Cr in some areas in Kosovo, mainly around the contamination sources, have also been reported by other authors. Borgna et al., (2009) determined high level of Cr from 24.9 to 5497 mg kg^{-1}.

Correlation of pseudo-total concentration among Ni and Cr (R^2=0.773) and Zn and Pb (R^2=0.7554), are given in figure 2.3. This type of correlation has not been noticed among other metals.



n= number of samples above the values

Figure 2. 3 Correlation between heavy metals

2.3.2 EDTA extractable (potential plant available) heavy metals in soil

Many authors consider EDTA a suitable extractant to assess the potential plant availability of most heavy metals for plant uptake (Norvell 1984; Kociałkowski et al., 1999; Kabala & Singh 2001, Damian et al., 2008).

The potential plant availability of heavy metals in soil was assessed using a "potential bioavailability factor (PBF)" calculated on the basis of the following equation:

$$PBF = \frac{M_{(EDTA)}}{M_{(AR)}} \text{x}100 \qquad (1)$$

Where M (AR) is the pseudo total metals concentration in soil and M(EDTA) is the EDTA extractable of metals in soil. On the basis of the PBF, Cu was extracted by EDTA in highest amount, from 5.72 to 57.92%, with a mean of 17.84% and median of 16.99% (Fig. 2.4). The lowest extraction efficiency was encountered for Cr, from 0.02 to 0.63%, mean of 0.12% and median of 0.098%. Other metals show the following mean values: Pb 17.63, Cd 23.48, Ni 6.88, Zn 6.71%. The median values are: Pb 16.27, Cd 15.3, Ni 6.84 and Zn 5.16% (Fig. 2.4). Extractability by EDTA follows this order: Cu ≈ Pb ≈ Cd >> Ni ≈ Zn >> Cr. Similar results have been reported by other authors (Ashraf et al., 2012; Abdu et al., 2012; Nanoni et al., 2011; Takac, et al., 2009; Mitsios 2005; Abollino et al., 2002). The influence of total content on the EDTA extracted amount of heavy metals is given in table 2.3. The content of organic matter and pH showed no significance for all metals.

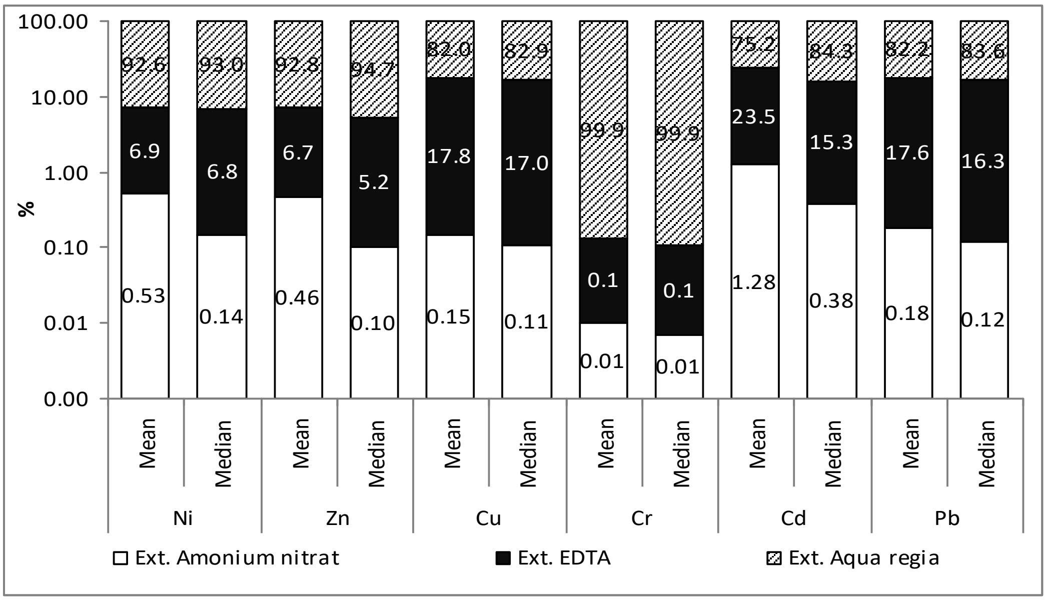


Figure 2. 4 The distribution of Ni, Zn, Cu, Cr, Cd and Pb in the differently extracted fractions.

Table 2. 3 The relationship between EDTA- extractable heavy metals and other properties

Regression equation								R^2
Ni_{EDTA} =	18.7	+	0.0227 Ni_{AR}	+	0.255 OM	-	2.26 pH_{H20}	0.616***
(T)	2.96**		13.5***		1.03		-2.27	
Zn_{EDTA} =	23	+	0.238Zn_{AR}	-	1.27 OM	-	3.07 pH_{H20}	0.882***
(T)	1.53		30.31***		-2.16		-1.55	
Cu_{EDTA} =	5.86	+	0.156Cu_{AR}	+	0.143 OM	-	0.881 pH_{H20}	0.352***
(T)	1.64		7.28***		0.99		-1. 53	
Cr_{EDTA} =	0.12	+	0.000216 Cr_{AR}	-	0.00124 OM	-	0.00731 pH_{H20}	0.348***
(T)	2.51		8.04***		-0.66		-0.97	
Cd_{EDTA}=	0.14	+	0.242 Cd_{AR}	-	0.0019 OM	+	0.004 pH_{H20}	0.169***
(T)	-0.01		4.98***		-0.05		0.03	
Pb_{EDTA} =	8.2	+	0.331Pb_{AR}	-	1.36 OM	-	1.66 pH_{H20}	0.889***
(T)	0.17		31.24***		-0.74		-0.22	

EDTA (metals are extracted with EDTA), AR (metals are extracted with *Aqua Regia*), T- Student's t-test
*, ** and *** indicate significant at 5, 1 and 0.1% confidence level, respectively.

2.3.3 Ammonium nitrate extractable (mobile) heavy metals in soil

The mobile fraction of heavy metals in soil was, analogous to PBF, assessed using a "mobile factor (MF)" calculated on the basis of the following equation:

$$MF = \frac{M_{(AN)}}{M_{(AR)}} \text{x}100 \qquad (2)$$

Where M(AR) is the pseudo-total metals concentration in soil and M(AN) is the ammonium nitrate extractable fraction of metals in soil. On the basis of MF, Cd was extractable in high percentages, up to 24.48%, with a mean of 1.28% and a median of 0.376% (Fig. 2.4). A lower percentage was encountered for Cr, from 0 to 0.02%, with a mean of 0.01% and a median of 0.007%. The other metals showed the following mean values: Ni 0.53, Pb 0.18, Cu 0.15 and Zn 0.46%. Median values are: Ni 0.144, Pb 0.122, Cu 0.11 and Zn 0.103% (Fig. 2.4). Thus, the range concerning the extractability of the different metals by NH4NO3 resulted as follows: Cd >>Ni>Pb>Cu>Zn>>Cr.

Table 2. 4 The relationship between NH4NO3- extractable heavy metals and other properties

Regression equation										R^2
Ni_{AN} =	4.27	+	0.00179 Ni_{AR}	+	0.0524 Ni_{EDTA}		0.0223 OM	-	0.609 pH_{H20}	0.622***
(T)	4.69***		4.89***		4.18***		-0.64		-4.31***	
Zn_{AN} =	2.87	+	0.000767Zn_{AR}	+	0.00855 Zn_{EDTA}	-	0.0078 OM	-	0.383 pH_{H20}	0.633***
(T)	6.60***		1.17		3.32***		-0.46		-5.58***	
Cu_{AN} =	0.0028	-	0.000354 Cu_{AR}	+	0.0018 Cu_{EDTA}	+	0.00361 OM	+	0.0027 pH_{H20}	0.037
(T)	0.04		-0.77		1.1		1.39		0.26	
Cr_{AN} =	0.0112	-	0.000004 Cr_{AR}	+	0.0159 Cr_{EDTA}	+	0.000016 OM	-	0.000737 pH_{H20}	0.061
(T)	2.88*		-1.46		2.21		0.11		-1.22	
Cd_{AN} =	0.0856	-	0.000749 Cd_{AR}	+	0.0235 Cd_{EDTA}	-	0.00024 OM	-	0.0114 pH_{H20}	0.801***
(T)	6.48***		-1.06		19.64***		-0.49		-5.77***	
Pb_{AN} =	1.22	-	0.000092Pb_{AR}	+	0.000313 Pb_{EDTA}	+	0.0119 OM	-	0.168 pH_{H20}	0.109**
(T)	4.17***		-0.47		0.56		1.04		-3.62***	

AN (metals are extracted with NH_4NO_3), AR (metals are extracted with *Aqua Regia*), EDTA (metals are extracted with EDTA), T- Student's t-test, *, ** and *** indicate significant at 5, 1 and 0.1% confidence level, respectively.

According to BBodSchV (1999) (0,1 mg kg^{-1}), 29% of all investigated samples exceeded the limit values for Pb, 6% for Ni (1.5 mg kg-^{1}), 3% for Zn (2 mg kg^{-1}), 1.5% for Cd (0.1 mg kg^{-1}) and 0.8% for Cu (1 mg kg^{-1}). Similar results have also been reported by other authors (Pueyo et al.; 2004; Mellum et al., 1998).

For the amount of heavy metals extracted with NH4NO3, the soil pH and the content of heavy metals extracted with EDTA (Table 2.4) played an essential role. Total metal content showed positive regression only for Ni at a confidence level of 0.1%.

Similar results were shown by Mellum et al., (1998), who did not find significance between the samples extracted with *aqua regia* and those extracted with DTPA. A positive regression with extracted metals by EDTA was determined for Ni, Zn and Cd (0.1% significance level), while there was no significance for Cr, Cu and Pb. The impact of pH on NH_4NO_3 extractable amounts was highly significant for Ni, Zn, Cd and Pb (0.1% significance level), whereas there was no significance for Cu and Cr.

2.4 Conclusions

In most of the soils under investigation total content of heavy metals in agricultural soils of Kosovo are within the permitted values given by the EU. Nevertheless 62% of all soil samples exceeded the limit value of total concentration of Ni. The potential bioavailable and mobile fractions of this element was determined at 6.84 and 0.144% respectively.

High concentrations of heavy metals have been noticed near contamination sources (mainly near metals' industries), which, in some cases, have exceeded many times the allowed limits. Moreover, the potential bioavailable form of metals around these sources has shown very high values, presenting a serious hazard for human health, since these soils are being cultivated and their products being consumed.

Cu has been the only metal which did not exceed the limit of permitted level for total concentration regarding EU standards.

The regression analysis has shown that the pseudo-total metal content significantly influenced ($p<0.001$) the amount of metals extracted with EDTA. The content of organic matter and the value of pH did not show any significance in the amount of extracted metals with EDTA.

Strong correlation ($p<0.001$) between soil pH value and mobile Ni, Zn, Cd and Pb has been shown. Correlation of potential bioavailable metal contents with mobile fractions did show significance ($p<0.001$) for Ni, Zn and Cd, as well as for Cr on a significance level of $p<0.05$. The pseudo-total metal content was significant ($p<0.001$) only for mobile Ni.

Based on the results of investigated soil samples and on the necessity for human health protection, the investigation on heavy metals concentrations in agricultural products of the Kosovo is an urgent and indispensable task and would fill a gap not met in the literature so far.

3

Plant uptake of metals, transfer factors and prediction model for two contaminated regions of Kosovo

Muhamet Zogaj and Rolf-Alexander Düring, 2016. Plant uptake of metals, transfer factors and prediction model for two contaminated regions of Kosovo, J. Plant Nutr. Soil Sci. 179, 630–640 DOI: 10.1002/jpln.201600022

Abstract

The bioavailability and plant uptake of heavy metals (HM), as well as finding the most reliable methods for the prediction of availability, continues to be one of the most crucial problems in agricultural and environmental studies. In agricultural soils from two regions in Kosovo known for its metal pollution, we collected 60 soil and plant samples (wheat, corn, potatoes and grass). Heavy metals were extracted from soil with aqua-regia (pseudototal concentration), NH_4OAc-EDTA (potential bioavailable) and NH_4NO_3 (mobile fraction), plant samples were digested with HNO_3/H_2O_2 (microwave assisted extraction). The pseudo total content of Cd, Pb and Zn showed high value in Mitrovice (mean: Cd - 2.92, Pb - 570.15 and Zn - 522.86 mg kg^{-1}), whereas in Drenas region Ni and Cr showed high value with a mean 258.54 and 203.22 mg kg^{-1}. Also, the potential bioavailability and mobile form of these metals were increased in Mitrovice (mean: Cd – 1.59, Pb - 217.05, Zn - 522.86 mg kg^{-1}, respectively Cd - 0.17, Pb - 0.64 and Zn - 15.45 mg kg^{-1}), compared to Drenas. Cd and Pb were elevated in potato tubers (mean Cd - 0.48 and Pb – 0.85 mg kg^{-1}). The TF was higher for micronutrients (Zn and Cu) than for non-essential metals (Cd and Pb). Multiple regression analysis showed a good model for prediction of Cd, Pb and Zn content in plant with significance 99.9%, whereas this model was not significant for Cu, Cr and Ni. Soil pH played a significant role in the content of Cd and Zn in wheat and potato plants. Clay content also showed significance in Cd concentration in wheat and potato plants, while carbon content was significant for Cd in grass plants, as well as for Zn in wheat and grass plants.

Key words: pseudototal concentrations / bioavailable fraction / plant uptake / multiple regression analysis

3.1 Introduction

Soil is the main source of micronutrients for plants, as well as of essential and nonessential metals. Elevated mobility and bioavailability of these elements can be a risk for the environment. Moreover, via plant uptake, high metal concentrations can adversely affect human health. Mobility and bioavailability of metals in soil are controlled by several factors, such as: pH, organic matter, clay content and cation exchange capacity (CEC) (Gandois et al., 2010; Groenenberg et al., 2010; Ivezić et al., 2012). The soil – plant transfer of metals is a part of chemical element cycling in nature (Kabata-Pendias, 2004). Further, metals may negatively affect plant growth as they influence such biochemical processes as metabolism, respiration, photosynthesis, and stomata opening (Samical et al., 2008).

The Kosovo, located in the center of Balkan Peninsula (N 43° 16' – 41° 53' and E 21° 16 – 19° 59'), represents a country of great interest for studies on behavior of metals in contaminated soils. In fact, mining and industrial activity mostly cause contamination of the environment and agricultural soils, which are located in different parts of eastern Kosovo. Some authors report high levels of heavy metals in areas near these contamination sources (Borgna et al., 2009; Nannoni et al., 2011; Šajn et al., 2013). Zogaj et al. (2014) identified some hotspots in agricultural soils, e.g. field sites near to the ore-metallurgic combine "Trepça" in Mitrovice, the ferronickel production plant "Ferronikeli" in Drenas, the battery Factory Ni-Cd in Gjilan and the mine of Kizhnica. Concentrations of metals in these hotspots have passed many times the target values and soil remediation intervention values regarding to Netherland standards (the New Dutch List, 2000).

To predict concentrations of heavy metals in plants due to uptake from soil or soil solution, mechanistic, empiric and mathematical models are used (Gupta and Aten, 1993; Rengel, 1993). The relationship between metal concentrations in various soil extracts and plants is often described by a transfer factor. Krauss et al. (2002) used Freundlich – type functions to predict Cd, Cu, Pb and Zn concentrations in wheat grain and leaf. Multiple regression analysis was used successfully by Ivezić (2011) to predict metal concentrations in wheat grain in uncontaminated soil. The aim of this research is to determine the concentration and bioavailability of heavy metals in soil, to describe their transfer into different plants, which are commonly cultivated in these regions, and to assess the influence of soil parameters using multiple linear regression analysis.

3.2 Materials and methods

3.2.1 Study area

The study area represents agricultural soils in two regions of Kosovo, that is Mitrovice region and the region of Drenas, which are considered the most polluted regions in Kosovo (Zogaj et al., 2014). Mitrovice region, which is located in the northern part of Kosovo was one of the main industrial sites of Former Yugoslavia and one of the most important mining districts in Europe (Nannoni et al., 2011). Within Mitrovice Industrial Park (Trepca) approximatly 40 mines, various concentrators, flotation and smelting plants, and several factories are included. This area has begun to be exploited intensively for the production of Pb, Zn, Au, Ag and Bi from the 1930s. The main industrial plants were the Zvecan Smelter and the Trepca battery factory, being located near agricultural and residential areas, which have produced large amounts of metals and created large volumes of waste accumulated in enormous dumps close to the plants. In 1989, it was estimated that the Zvecan smelter emitted 730 t/year of particulate matter, as well as 438, 83, 3.6 t/year of Pb, Zn, and Cd, respectively (Frese et al., 2004). The agricultural land is developed along the valleys of Sitnica and Ibri rivers close to Mitrovica Industrial Park. The main cultivated crops are wheat, maize, potato and some vegetables. The climate in this region is continental, with warm summers and cold winters. Air temperatures range from –20°C to +35 °C. The main annual rainfall is about 650 mm (Mehmeti et al., 2010).

The Drenas region lies in the central part of Kosovo. The main industry in this part is Ferronikeli factory, which is very close to agricultural and residential areas. It started work in 1984 and was projected for processing 1 374 000 t of ore a year and the production of 52 000 t of iron-nickel (Haxhiaj and Haxhiaj, 2012). After decades of operation, around 3 millions of tons of granulated slag are stored in dump close to the factory. This slag is very light material with a low density (Veliu et al., 2009), and this material might be spread around by wind or water erosion.

The mid-continental climate in the Drenas region is characterized by cold winters and hot summers. Air temperatures range from –18°C to +36 °C and rainfall is about 670 mm (Mustafa et al., 2012).

3.2.2 Soil and Plant Sampling

In total, 60 topsoil samples (0-30 cm depth in arable land and 0-5 cm in meadow) were collected throughout the agricultural area in the two mentioned regions of Kosovo (30 samples per region), according to the random method (BBodSchV, 1999; Theocharopoulos et al., 2001; ICP Forest, 2006). Each soil sampling has been prepared out of 10 sub samples that have been taken with a distance of 10-50 m between them (depending on size and shape of the plots) using a hand auger. At the same time and at the same places, there have also been collected plant samples, that is, 20 wheat, 20 maize, 10 potato and 10 grass samples. All samples were collected shortly before harvest in July and September 2012.

3.2.3 Sample analysis

Soil samples were dried at room temperature, aggregates were crushed and sieved (a < 2 mm). Plant samples were washed with distilled water and dried at a temperature 50 °C. The samples were divided into vegetative and generative parts, then ground and sieved (a < 1 mm). Both soil and plant samples were stored at room temperature until analysis. Soil pH was measured in 0.01 M $CaCl_2$ with a ratio of 1:2.5 (DIN ISO 10390 2005). The total amount of carbon (C) and nitrogen (N) was determined by gas-chromatography using a C-N-S element analyzer (Elementar). Particle size distribution was determined by a combined sieving and pipette method after decomposition of carbonates (HCl) and organic matter (H_2O_2) and dispersion in Na-pyrophosphate (DIN EN ISO 14688 – 1: 2003-01 2003)

The pseudo total contents of heavy metals in soil were extracted with *aqua regia* using microwave assisted extraction (MAE) (6 ml of 35% HCl and 2 ml of 65% HNO_3) from finely ground samples (US EPA 3051A). As this pseudo total content is insufficient to determine ecotoxicologically relevant heavy metals, the exchangeable and mobile fractions of heavy metals (potentially plant available and easily leachable) were assessed by extraction with NH_4OAc-EDTA (ammonium acetate and ethylenediaminetetraacetic acid) and 1M NH_4NO_3 (DIN 19730 2009) according to German regulation (BBodSchV 1999). The total content of metals in plants was extracted using MAE (5 ml H_2O, 5 ml HNO_3 and 3 ml H_2O_2) (Czarnecki and Düring, 2014).

Metal concentrations in soil and plant extracts were measured by an inductively coupled plasma optical emission spectrometer (ICP-OES; Varian 720ES). Very low concentrations of Cd in soil extracts were determined by graphite furnace AAS (Perkin Elmer SIMAA 6000).

The quality assurance of the analytical method for soil samples were examined by analyzing two certified reference materials (CRMs), “7001” (light sandy soil) supplied by Analytica Co. Ltd, Prague, Czech Republic, and “ISE 918”, (sandy soil) supplied by the WEPAL (Wageningen), and for plant samples a certified reference material (CRM), “IPE 151”, (poaceae/grass material) supplied by WEPAL (Wageningen).

3.2.4 Statistical analysis

Descriptive statistics, analysis of variance (ANOVA), comparison of means, correlation, regression and principal component analysis were conducted using SPSS 22.0 software package for Windows. Analysis of variance and Student T-test was done to determine significant differences and the influence of different regions on heavy metals in soils and plants. Pearson correlation coefficients were determined to view and prevent multicollinearity of the parameters analyzed. Then, the principal component analysis was used to determine sampling adequacy (KMO and Bartlett's Test). Further, multiple linear regression analysis was used to derive models so to predict concentrations of heavy metals in plants.

3.3 Results and discussion

3.3.1 Heavy metals in agricultural soils

Soil samples were analyzed for parameters which influence the behavior of metals in soil, like: pH, clay, carbon (C) and nitrogen (N) content. Student t-test was used in order to assess the impact of the region on the parameters analyzed. Descriptive statistics showed that the mean of pH and clay content was approximately the same in the two regions. Contents of C and N showed different mean (2.3 and 1.6, 0.22 and 0.18%, respectively) between regions and student T-test showed highly significant difference ($p<0.001$) for C content and significant difference for N content ($p<0.05$). Significant differences of C and N contents are due to the influence of meadow soil samples (10 meadow samples in Drenas out of a total sample number of 30) taken from the first region (Table 3.1).

Tabela 3. 1 Statistical description of soil properties in the two regions (n=60)

	pH		Clay %		C %		N %	
	Drenas	Mitrovice	Drenas	Mitrovice	Drenas	Mitrovice	Drenas	Mitrovice
Mean	6.07	5.91	38.03	38.09	2.33	1.61	0.22	0.18
Median	6.09	5.88	35.06	37.78	1.91	1.60	0.19	0.18
Std. Dev.	0.73	0.54	12.97	8.40	1.07	0.28	0.09	0.02
Minimum	4.54	4.78	17.22	22.55	1.11	1.18	0.11	0.14
Maximum	7.38	6.97	68.45	57.28	5.04	2.22	0.47	0.24
T-test	0.955^{ns}		-0.020^{ns}		3.558***		2.460*	

ns - no significant difference; *, ** and *** indicate significant difference at 5, 1 and 0.1% confidence level, respectively.

3.3.1.1 Pseudo total content of metals

In Fig. 3.1, metal concentrations are given as box-plots. For some regions and some metals, increased mean values compared to the median values indicate an uneven distribution of the concentration of Cd, Cr, Ni, Pb and Zn. In Mitrovice, Cd concentrations are increased up to 10.3 $mg{\cdot}kg^{-1}$ and Pb and Zn show severely increased concentrations at some sampling sites (up to 1450 and 1700 $mg{\cdot}kg^{-1}$ respectively). For the region of Drenas we found some sites with Cr and Ni concentrations up to 626 and 1560 $mg{\cdot}kg^{-1}$ respectively, which may be a result of the emissions of the factory “Ferronikel”.

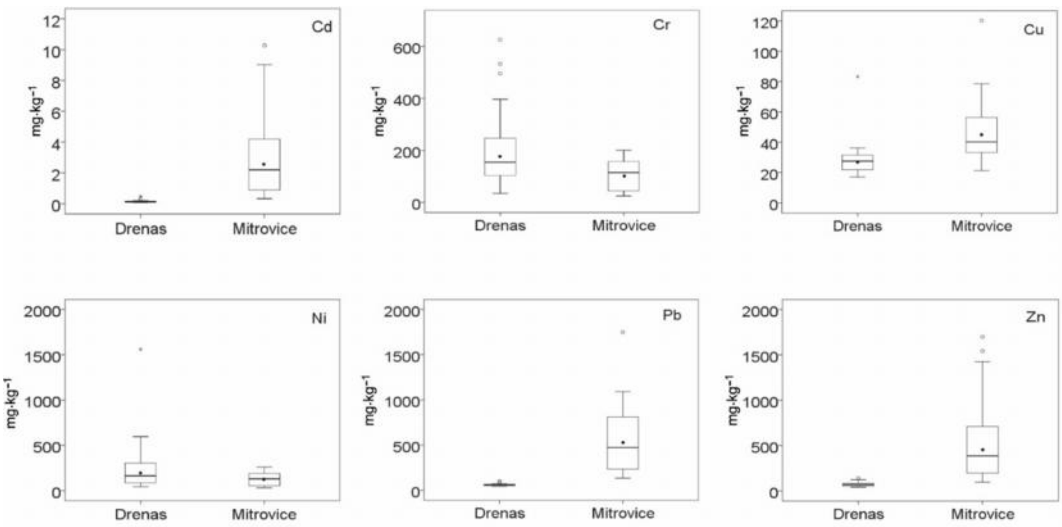


Figure 3. 1 Concentrations of pseudo total metals (AR) in agricultural soils in two regions of Kosovo, (box-plots indicate: minimum, first quartile, mean, median, third quartile, maximum below upper fence and outliers with maximum observation) (n=60)

Student T-test showed significant differences between regions for all investigated elements on a significance level of $p<0.001$ except for Ni concentrations were different on significance level of $p<0.05$. This can be interpreted as a result from the different metallurgic industries developed in the regions.

3.3.1.2 Potential plant availability (EDTA extractable) of heavy metals

EDTA is considered a suitable extractant to assess the potential plant availability of most heavy metals for plant uptake (Kociałkowski et al., 1999; Kabala and Singh, 2001, Damian et al., 2008).

The “potential bioavailability factor (PBF)” (Bielicka-Giełdoń et al., 2013) was used to assess potential plant availability of heavy metals in soil, calculated on the basis of the following equation:

$$PBF = \frac{M_{(EDTA)}}{M_{(AR)}} x100 \quad (1)$$

Where M(AR) is the pseudo total metal concentration in soil and M(EDTA) is the EDTA extractable portion of metals in soil. On the basis of the PBF, Cd showed highest potential bioavailability, from approx. 50% to more than 80%, in Drenas region, and from 35% to more than 70% in Mitrovice region. Also, Asami (1984) found that half or more of the total Cd in Japanese paddy soil is exchangeable and available to plants. The lowest extraction efficiency was encountered for Cr, from 0.02 to 0.13%, with a mean of 0.05, in Drenas region, and from 0.02 to 0.08% (mean 0.06) in Mitrovice region (Fig. 3.2). Cr occurs in soils mainly (>80% of total content) in the immobile residual fraction and is hardly mobile, thus not easily available to plants (Kabata-Pendias, 2011). The low EDTA – extractability of Cr is confirmed in other studies (Megremi, 2009; Cappuyns, 2012). Megremi (2009) reports only 0.1% of Cr was extracted by EDTA in polluted soils. Extractability by EDTA follows this order in Drenas region: Cd > Pb > Cu > Ni > Zn >> Cr, and in Mitrovice region: Cd > Pb > Cu > Zn > Ni >> Cr. Similar results have been reported in other studies (Takac, et al., 2009; Abdu et al., 2011; Nannoni et al., 2011; Ashraf et al., 2012; Zogaj et al., 2014).

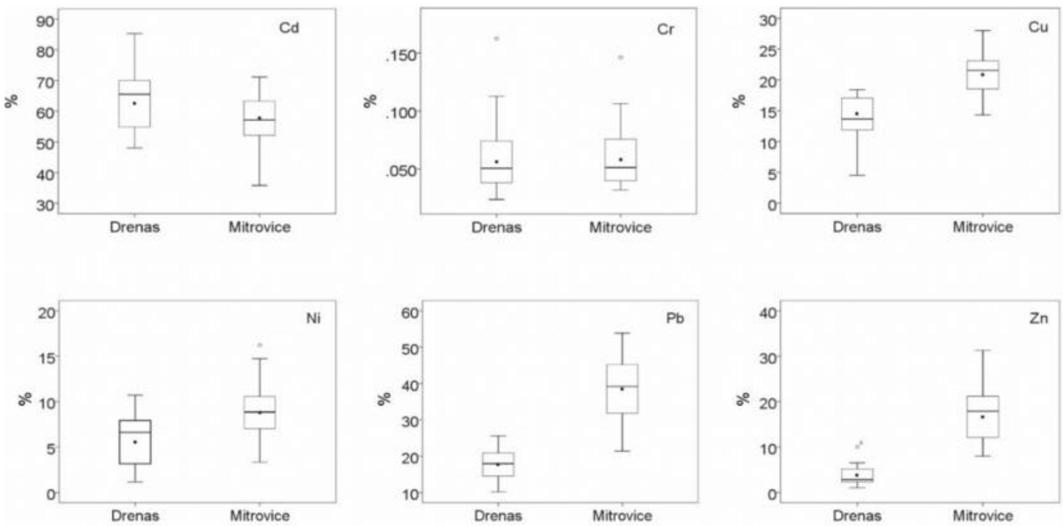


Figure 3. 2 PBF (Potential plant availability factor) of heavy metals in agricultural soils in two regions of Kosovo (% EDTA extractable from AR extractable) (box-plots indicate: minimum, first quartile, mean, median, third quartile, maximum below upper fence and outliers with maximum observation) (n=60)

In addition, Student t-test showed highly significant differences of EDTA extractable metal concentrations between regions, except the Ni concentrations which did not differ between the two regions. All other elements showed differences between regions on a high significance level ($p<0.001$).

3.3.1.3 Mobile form (Ammonium nitrate extractable) of heavy metals

Ammonium nitrate is used to assess the mobile form of heavy metals in soil (Gryschko et al., 2004; Sabienë et al., 2004; Abdu et al., 2012). In Germany, exchangeable (mobile) soil metals are estimated by a 1M NH_4NO_3 extraction procedure (DIN 19730, 2009; BBodSchV, 1999).

The "Mobility factor (MF)" was, analogous to PBF, used to assess mobile fraction of heavy metals in soil, calculated on the basis of the following equation:

$$MF = \frac{M_{(AN)}}{M_{(AR)}} x100 \tag{2}$$

Where M(AR) is the pseudo total metal concentration in soil and M(AN) is the ammonium nitrate extractable portion of metals in soil.

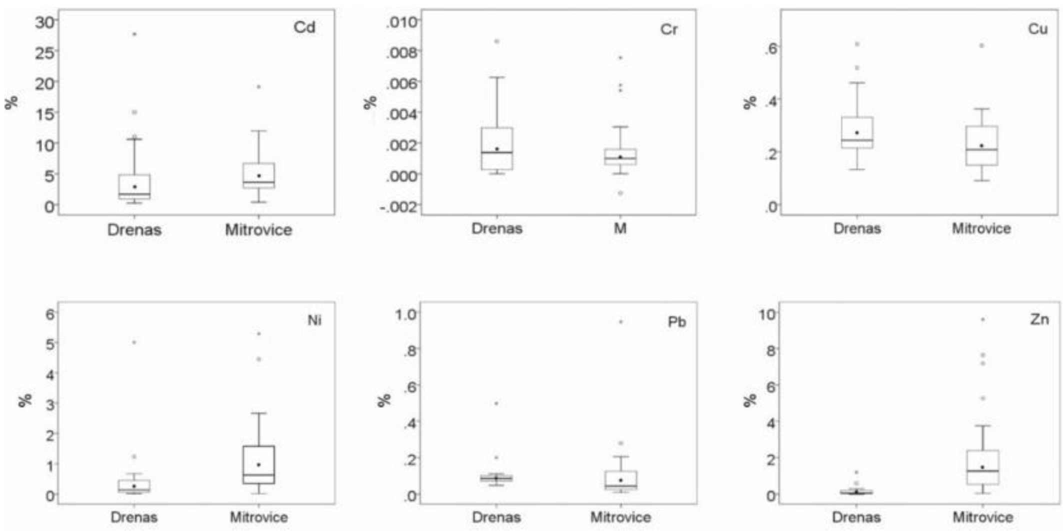


Figure 3. 3 MF (Mobility factor) of metals in agricultural soils in two regions of Kosovo (% NH4NO3 extractable from AR extractable) (box-plots indicate: minimum, first quartile, mean, median, third quartile, maximum below upper fence and outliers with maximum observation) (n=60)

On the basis of the MF, Cd showed highest mobility, from 0.25 to more than 25%, in Drenas region, and from 0.4 to approx. 20% in Mitrovice region. The lowest extraction efficiency was encountered for Cr, up to 0.006%, in Mitrovice region, and up to 0.009% in Drenas region. Extractability by NH_4NO_3 follows this order in Drenas region: Cd > Ni > Cu > Zn > Pb > Cr, and in Mitrovice region: Cd > Zn > Ni > Cu > Pb >> Cr.

Student T-test showed highly significant differences of ammonium nitrate extractable metal concentrations in soils between regions for Cd ($p<0.001$), highly significant differences for Zn ($p<0.01$) and significant differences for Cr and Pb ($p<0.05$), whereas Cu and Ni concentrations did not differ between the two regions.

3.3.2 Metals in plants and transfer factor

3.3.2.1 Metal contents in plants

Metal concentrations in different plants are shown in Table 3.2. The concentrations of Cd, Cr, Ni, Pb, in wheat were higher in vegetative than in generative parts in both regions. However Cu and Zn were higher in generative than in vegetative parts, similar to data previously reported by Puschenreiter and Horak (2000). In corn all metal concentrations were higher in vegetative than in generative parts. In general metal concentrations in analyzed plants showed comparable values with reported values by several European countries (Kabata-Pendias, 2011), except for Pb in potato tubers, which exceeded the limit of the permitted level for Pb content in foodstuff regarding EU standard (Commission Regulation (EC) No 1881/2006). In addition, some potato samples showed concentrations of Cd above the permitted level. On the one hand this indicates a risk due to metal transfer into food. On the other, EC 1881/2006 is targeting on peeled potato, while we analyzed it unpeeled. Therefore further assessment should be done to deny or approve this possible risk.

The Pb and Cd uptake by plants depends on several factors, such as availability, pH, SOM, clay content, CEC, as well as genetic plant factors, root surface area, and root exudates. The availability of Cd and Pb in soils, mainly in Mitrovice region, which is considered contaminated, may enhance the plant uptake of these metals.

Tabela 3. 2 Concentrations of metals in vegetative and generative parts of different plants (mg.kg-1 dry weight). Comparison for wheat and corn between the two regions Drenas and Mitrovice

plants	regions		Cd		Cr		Cu		Ni		Pb		Zn	
			VP	GP	VP	GP	VP	GP	VP	GP	VP	GP	VP	GP
Wheat (n=20)	Drenas	Mean	.07	.00	3.79	.08	3.10	6.22	5.80	1.39	.38	.00	7.16	30.19
		Min.	.04	.00	1.84	.05	2.45	5.33	2.65	.77	.02	.00	3.90	24.77
		Max.	.12	.00	8.57	.13	4.20	7.25	8.99	1.96	1.23	.00	11.76	48.89
		STDEV	.02	.00	1.90	.03	.51	.66	2.09	.44	.36	.00	2.56	7.35
	Mitrovice	Mean	.48	.15	2.83	.08	3.20	6.67	3.36	1.50	2.73	.00	43.11	52.89
		Min.	.12	.01	1.29	.00	2.59	5.68	2.08	.57	.63	.00	13.37	31.38
		Max.	1.45	.50	7.04	.19	3.86	7.78	8.08	4.35	8.94	.00	155.55	85.10
		STDEV	.42	.16	1.57	.06	.51	.78	1.74	1.12	2.38	.00	42.93	15.33
		T-Test	-3.06**	-2.86**	1.23ns	0.36ns	0.45ns	-1.39ns	2.84*	-0.272ns	-3.08**	-	-2.64*	-0.42***
Corn (n=20)	Drenas	Mean	.14	.00	3.27	.05	7.73	2.63	9.51	2.45	.29	.00	48.59	22.32
		Min.	.06	.00	1.97	.00	5.29	1.52	5.56	1.31	.14	.00	33.09	18.41
		Max.	.33	.00	4.79	.14	12.82	4.00	15.93	4.34	.42	.00	83.87	28.22
		STDEV	.08	.00	.83	.04	2.26	.83	3.99	1.07	.09	.00	16.71	3.44
	Mitrovice	Mean	1.06	.01	2.56	.05	7.29	2.77	8.52	1.86	4.24	.28	206.57	33.56
		Min.	.09	.00	1.85	.00	5.06	1.89	3.20	.81	.63	.00	86.23	24.31
		Max.	4.43	.10	3.05	.33	12.72	4.21	20.27	4.45	22.03	2.58	522.87	46.34
		STDEV	1.24	.03	.46	.10	2.41	.71	6.23	1.09	6.48	.81	158.85	7.72
		T-Test	-2,33*	-1,32ns	2,36*	-0.32ns	0.48ns	-0.42ns	0.43ns	1.21ns	-1.93ns	-1.11ns	-3.13**	-4.21***
Potato[a] (n=10)	Mitrovice	Mean	.48		.47		10.55		4.78		.85		33.96	
		Min.	.18		.26		7.79		3.40		.06		18.30	
		Max.	1.41		.75		13.76		7.10		2.43		64.65	
		STDEV	.36		.14		2.07		1.38		.84		13.51	
Grass (n=10)	Drenas	Mean	.10		6.06		6.05		16.18		.99		23.84	
		Min.	.06		2.91		4.31		7.65		.63		16.21	
		Max.	.17		10.80		14.21		30.08		1.57		46.34	
		STDEV	.03		2.50		2.93		6.38		.32		8.50	

VP-vegetative parts; GP-generative parts; a-unpeeled potato tubers;
ns - no significant difference; *, ** and *** indicate significant difference at 5, 1 and 0.1% confidence level, respectively.

When Pb is present in soluble forms in soil solution, plant roots are able to take up great amounts of this metal, and the uptake rate increases with increasing its concentration in the solution and with time. In many publications on this topic, soil pH and clay content are listed as the major soil factors controlling both total and relative uptake of Cd (Kabata-Pendias, 2011). Metal solubility tends to increase at lower pH and decrease at higher pH values. In well aerated (oxidizing) acid soils, Cd is mobile and available to plants, while in poorly aerated (reducing) neutral or alkaline soils, is less available (Kabata-Pendias, 2004). Bingham et al. (1980) found that the Cd content of rice grain is highly dependent upon the soil pH and is the highest at pH 5.5. The ability of the clays to bind the metal ions is correlated with their cation and anion exchange capacity (CEC, AEC) (Kabata-Pendias, 2011). Eriksson (1989) found that Cd was more soluble and plant available in sandy soil than in clay soil for a given total Cd concentration.

3.3.2.2 Transfer factor from soil to plants

The transfer of heavy metals from soil to plant (transfer factor-TF) was used to determine the relative uptake of heavy metals by plants (Gupta et al., 2008), calculated on the basis of the following equation

$$TF = \frac{M_P}{M_S} \tag{3}$$

Where Mp is the metal concentration in plant tissue and Ms is the EDTA extractable metal in soil. Based on this calculation and given in Table 3, transfer of Cr seems to be rather high with TF from 23 to 142 in vegetative parts of corn in Mitrovice region. But this could be fully drawn back to the very low EDTA extractability of Cr, resulting in these high TFs. Other metals had these TF: Zn 17 – 74, Ni 0.1 – 10, Cu 0.15 – 9, Cd 0.1 – 8 and Pb up to 0.4. TF in vegetative parts follows this order: Cr >> Zn > Ni > Cu > Cd >> Pb. In generative parts Zn showed the highest TF from 0.1 to 155 and Pb showed the lowest TF up to 0.005. Other metals had these TF: Cu 0.1 – 17, Cr up to 16, Ni 0.07 – 2.5 and Cd up to 1.4. TF in generative parts follows this order: Zn >> Cu > Cr >> Ni > Cd >> Pb.

Tabela 3. 3 Transfer factors for metals from soil to vegetative (TFSV) and generative parts (TFSG) and differences between regions

Plant	Region		Cd		Cr		Cu		Ni		Pb		Zn	
			TFSV	TFSG	TFSV	TFSG	TFSV	TFSG	TFSV	TFSG	TFSV	TFSG	TFSV	TFSG
Wheat (n=20)	Drenas	Mean	0.8	0.0	68.09	1.53	0.87	1.7	0.97	0.25	0.04		3.8	15.5
		Range	0,4 – 8.0	0.0	11-164	0.5-3.7	0.5-8.7	1.1-17.2	0.1-9.7	0.04-2.5	0.002-0.4		1.5-38	8-155
	Mitrovice	Mean	0.54	0.14	53.8	1.56	0.49	1.03	0.43	0.19	0.02		0.88	1.69
		Range	0.2 - 5.4	0.03 - 1.4	30-540	0-15.7	0.15-5	0.4-10	0.2-4.3	0.07-2	0.005-0.2		0.3-9	0.4-17
	T-Test		1.77^{NS}		0.85^{NS}	-0.06^{NS}	3.34**	3.26**	3.12**	0.87^{NS}	1.83^{NS}		4.09***	-4.2***
Corn (n=20)	Drenas	Mean	2.07	0.0	42.95	0.66	2.5	0.86	1.27	0.3	0.04	0	37.9	17.1
		Range	0.8-4	0.0	18-62	0-1.8	1-5.3	0.3-2	0.18-3	0.1-0.7	0.02-0.06	0	17.3-74	9-30
	Mitrovice	Mean	0.43	0.003	68.2	0.94	0.67	0.29	1.57	0.3	0.013	0.001	2.14	0.53
		Range	0.19-1	0-0.02	24-142	0-5.5	0.35-1.5	0.09-0.94	0.2-3.5	0.07-0.7	0.003-0.04	0-0.005	0.7-10.8	0.09-3
	T-Test		5.28***		-1.94^{NS}	-0.53^{NS}	4.85***	3.82***	-0.59^{NS}	0.04^{NS}	4.34***		5.92***	8.46***
Potato (n=10)	Mitrovice	Mean	0.47		10.67		1.26		0.51		0.004		0.72	
		Range	0.1-0.86		5.7-22		0.4-2		0.2-1.5		0-0.006		0.17-2	
Grass (n=10)	Drenas	Mean	0.98		68.2		1.8		1.64		0.096		8.2	
		Range	0.51-1.6		18.9-132		0.8-3.5		0.4-3		0.06-0.2		3.2-20	

ns - no significant difference; *, ** and *** indicate significant difference at 5, 1 and 0.1% confidence level, respectively.

According to Kabata-Pendias (2011), Cr is poorly soluble in soil solution and not easily taken up by plants; Pb is relatively strongly sorbed by soil particles and not readily transported to above-ground parts of plants; Cu, Ni are mobile in soil and readily taken up by plants; Cd, Zn are very mobile in soil and easily bioaccumulated by plants and our results do confirm this argument. In general, TF was higher in soil samples with low content of metals, compared to soil samples with high content of metals. The higher TF for various crop species in uncontaminated soil compared to contaminated soil are also reported by Lübben (1993, Cited by Puschenreiter and Horak, 2000). Therefore, we suggest to be careful when using this factor in the future, as it seems to depend on the concentration level in soil. This means, from contaminated soils nonessential metals may be taken up with a relatively small TF, but in absolute numbers the concentration of the plant would be high and therefore can be dangerous for human health.

3.3.2.3 Model to predict metal content in plants

Since it is well known that soil properties play an important role in plant availability of metals, we used multiple regression analyses to predict metal concentrations in plants. The model for multiple linear regressions, given n observations, is

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p + \varepsilon \quad (4)$$

where error term ε is uncorrelated with $x_1, x_2, \ldots, x_p$ and is distributed with mean zero and variance σ^2. The total standardized variance (R^2) in a population explained by the model in equation (4), described by Zumbo (2007), can be written as

$$R^2 = \sum_j \beta j \rho j \quad (5)$$

where βj is the standardized regression coefficient corresponding to xj, and ρj is the simple correlation (i.e., zero-order correlation) between y and xj. The relative explanation index (REI) of variance (x_i explained by f_k) is used to determine the impact of soil properties, which is calculated based in the equation (Yanai and Ichikawa, 2007)

$V_{ik} = Var\ (x_i)\ \{Corr\ (x_i,f_k)\ \}^2$ (6)

where Corr (x_i,f_k) indicates the correlation coefficient between x_i and f_k.

As we determined high multicollinearity for some soil parameters and metal fractions, by principal component analyses and after data reduction, we limited the discussion to pH, clay, organic carbon, and EDTA extracts of metals.

Tabela 3. 4 Prediction model of metal contents in vegetative (VP) and generative parts (GP) of plants from backward procedure

Plants	Variables in equation[a]	**R^2**
Wheat (n=20)		
Cd $_{VP}$	$Y = 1.345 - 0.494X_1 + 0.307X_2 + 0.729X_4$	0.779***
Cd $_{GP}$	$Y = 0.457 - 0.477X_1 + 0.303X_2 + 0.726X_4$	0.764***
Pb $_{VP}$	$Y = 0.281 + 0.838X_4$	0.702***
Zn $_{VP}$	$Y = 66.985 - 0.19X_1 + 0.879X_4$	0.865***
Corn (n=20)		
Cd $_{VP}$	$Y = 0.077 + 0.735X_4$	0.540***
Cd $_{GP}$	$Y = -0.002 + 0.539X_4$	0.290*
Ni $_{GP}$	$Y = 1.331 + 0.570X_4$	0.324**
Pb $_{VP}$	$Y = -0.617 + 0.702X_4$	0.492***
Pb $_{GP}$	$Y = -0.086 + 0.473X_4$	0.224*
Zn $_{VP}$	$Y = 44.416 + 0.943X_4$	0.883***
Zn $_{GP}$	$Y = 23.585 + 0.824X_4$	0.679***
Potatoes (n=10)		
Cd	$Y = 0.651 - 0.449X_1 + 0.653X_2 + 0.623X_4$	0.837**
Cr	$Y = -0.408 + 0.576X_1$	0.332[NS]
Cu	$Y = -0.773 + 0.735X_3$	0.541*
Ni	$Y = -1.145 + 0.577X_3$	0.333[NS]
Pb	$Y = -0.413 + 0.956X_4$	0.915***
Zn	$Y = 58.051 - 0.231X_1 + 0.963X_4$	0.969***
Grass (n=10)		
Cr	$Y = 1.508 + 0.689X_2$	0.474*
Ni	$Y = 3.091 + 0.778X_2$	0.605**
Zn	$Y = 4.974 + 00748X_3$	0.559*

[a] X_1 (soil pH), X_2 (soil clay content), X_3 (soil carbon content), X_4 (metal bioavailability-EDTA extract)
*, ** and *** indicate significance at 5, 1 and 0.1% confidence level, respectively, [NS] no significance.

Generally, metal extraction by EDTA can be indicative for concentration of metals in plants. In Table 3.4 the presented parameters, mainly explain the concentrations of metals in our analyzed plant samples. In wheat plants (for both vegetative and generative parts) Cd, Pb and Zn concentrations could be explained highly significant ($p < 0.001$) by the chosen parameters with R^2 values higher than 0.7. For the other metals analyzed, this model did not significant correlation and was not able to explain plant concentration. Our results are in line with the reported results from Krauss et al. (2002) and Ivezić (2011). EDTA extractable Cd is a major factor influencing Cd concentrations in wheat straw and grain, which has contributed 51.84% in relative explanation index (REI) of variance. Also pH and clay played a significant role, as pH contributed with 17.5% in straw and 16.3% in grain, and the REI for clay was 6.8% and 6.7%. EDTA extractable Pb played a significant role in REI with 70.2% for Pb content in wheat straw. REI-s for Zn were: Zn-EDTA 75.2%, pH 3.5% in straw, Zn-EDTA 68.9%, pH 15.9 and C 12.7 in grain wheat (Fig. 3.4a).

In the prediction of metal contents in corn, both in vegetative and generative parts, exclusively metal EDTA extraction plays a significant role (R^2 values from 0.22 to 0.88). So, prediction model for Cd, Pb, Zn in plant and Zn in grain showed high significance ($p<0.001$). For Ni in grain the significance level was 99% ($p<0.01$), and for Cd, Pb in grain the significance level was 95% ($p<0.05$). The prediction of Cr in plant was not significant. The REI was for Cd 54% in plant and 29% in grain, Ni in grain 32.5%, Pb in plant 49.3% and in grain 22.4%, Zn in plant 88.9% and in grain 67.9% (Fig. 3.4b).

The prediction model for metal concentration in potato tubers showed high significance ($p<0.001$) for Pb and Zn (R^2 values from 0.92 to 0.97), significance ($p<0.01$) for Cd (R^2: 0.84), and significance ($p<0.05$) for Cu (R^2: 0.54). Cr and Ni didn't show any significance at level 95% (Table 3.4). Regarding Cd contents, Cd-EDTA extraction had contributed with 17.9% followed by clay with 15.4 and pH with 11.5% in REI. The REI for Cu content was significant only for C, which contributed 54%, while for Pb, Pb-EDTA extract contributed 91.4%. Zn contents in potatoes were significantly explained by Zn-EDTA extract and pH with 92.7 and 5.3% respectively (Fig. 3.4c).

Concerning the grass plant metal content, the model explained significantly Ni at level 99% and Cr and Zn at level 95% (Table 3.4). Clay content was significant for Cr and Ni with 47.5 and 60.5% contribution in REI. While, C content played a significant role in plant Zn content with REI 55.9% (Fig. 3.4d).

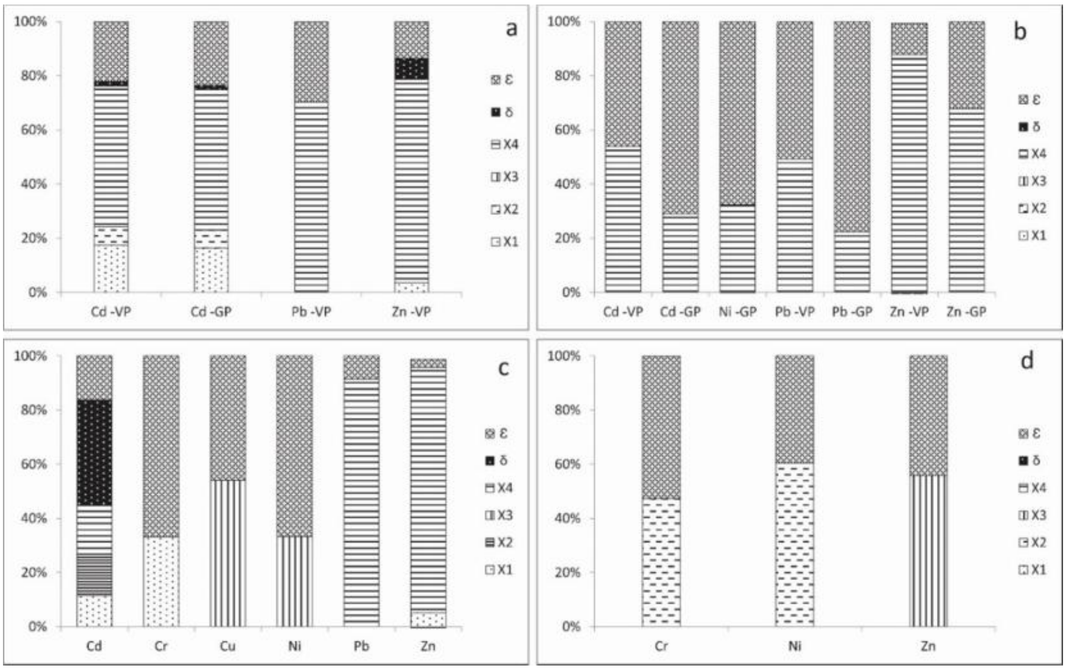


Figure 3. 4 Relative explanation index (REI) of variance (significance level ≥ 95%), relationship between heavy metals content in plant and other properties (X1 soil pH, X2 soil clay content, X3 soil carbon content, X4 metals bioavailability-EDTA extract, δ overlap term, ε error term). a wheat plant, b corn plant, c potato tubers and d grass plant.

3.4 Conclusions

The pseudo total concentrations of Cd, Pb and Zn in different agricultural soils of Kosovo in the Mitrovice region and Ni in both Mitrovice and Drenas region were significantly higher than the target values for these metals according to Netherland standards (the New Dutch List, 2000). The bioavailability and mobile form (EDTA extraction and NH_4NO_3 extraction) for Cd, Pb and Zn were also significantly higher in Mitrovice region. The concentration of Cd in potato tubers in Mitrovice region indicates a risk due to metal transfer into food, where high amounts of it were also found in wheat grain. Further, Pb was found in high amounts in potato tubers and corn grain, but not detected in wheat grain in Mitrovice region.

Although bioavailability of metals was high, the TF (as ratio of metal concentrations in plant to EDTA-extractable) from soil to plant was higher for micronutrients (Zn and Cu) than non-essential metals (Cd and Pb). Further, TF was higher in vegetative than generative parts for toxic metals and conversely for micronutrients. So, we suggest to be careful when using this parameter any longer, because it seems not suitable to assess a risk for plant cultivation. In our investigation, some plants with high concentrations of toxic metals showed lower TF compared to other samples of the same plant species with lower content of toxic metals.

The multiple regression analysis was a good model to predict Cd, Pb and Zn concentrations in wheat, corn, and potatoes. However, one should be careful when interpreting the model results, as the R^2 values for some metal – plant combinations are rather low (i.e. below 0.5 which means that only 50% of variance could be explained). Soil pH played a significant role for the uptake of Cd and Zn in wheat and potato plants, and the control of soil pH is a key to control the availability of these metals. Additionally, clay content was a significant impact on the concentration of Cd in wheat and potato plants, while the C content of the soil was significant for Zn in wheat and grass plants, as well as for Cd in grass plants.

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Declaration

I declare: this dissertation submitted is a work of my own, written without any illegitimate help by any third party and only with materials indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At any time during the investigations carried out by me and described in the dissertation, I followed the principles of good scientific practice as defined in the "Statutes of the Justus-Liebig-University Giessen for the Safeguarding of Good Scientific Practice".

Giessen, November 2016